TRANSMISSION ELECTRON MICROSCOPIC STUDY OF THE MICROVASCULATURE OF THE HUMAN POSTPARTUM FALLOPIAN TUBE

Dissertation submitted for

M.D Anatomy Branch V

Degree Examination

The Tamil Nadu Dr. M.G.R Medical University

Chennai, Tamil Nadu

April 2015

CERTIFICATE:

This is to certify that the dissertation entitled **"Transmission electron microscopic study of the microvasculature of the human postpartum Fallopian tube"** is a bona fied work of **Dr. Minu Rekha B.** in partial fulfilment of the requirements for the M.D Anatomy examination (Branch V) of The Tamil Nadu Dr. M.G.R Medical University, Chennai to be held in April 2015.

DR. BINA ISAAC, M.S.,
Professor and Head,
Department of Anatomy,
Christian Medical College,
Vellore, Tamil Nadu. Signature

DR. ALFRED JOB DANIEL, M.S.,
Principal,
Christian Medical College,
Vellore, Tamil Nadu. Signature

CERTIFICATE:

This is to certify that the dissertation entitled **"Transmission electron microscopic study of the microvasculature of the human postpartum Fallopian tube"** is a bona fied work of **Dr. Minu Rekha B.** in partial fulfilment of the requirements for the M.D Anatomy examination (Branch V) of The Tamil Nadu Dr. M.G.R Medical University, Chennai to be held in April 2015.

Guide:

DR. J. SUGANTHY,
Professor,
Department of Anatomy,
CMC, Vellore. Signature

Co-guides:

DR. SANTOSH JOSEPH BENJAMIN,
Assistant professor,
Department of Obstetrics and Gynaecology,
CMC, Vellore. Signature

DR. VISALAKSHI JEYASEELAN,
Lecturer,
Department of Biostatistics,
CMC, Vellore. Signature

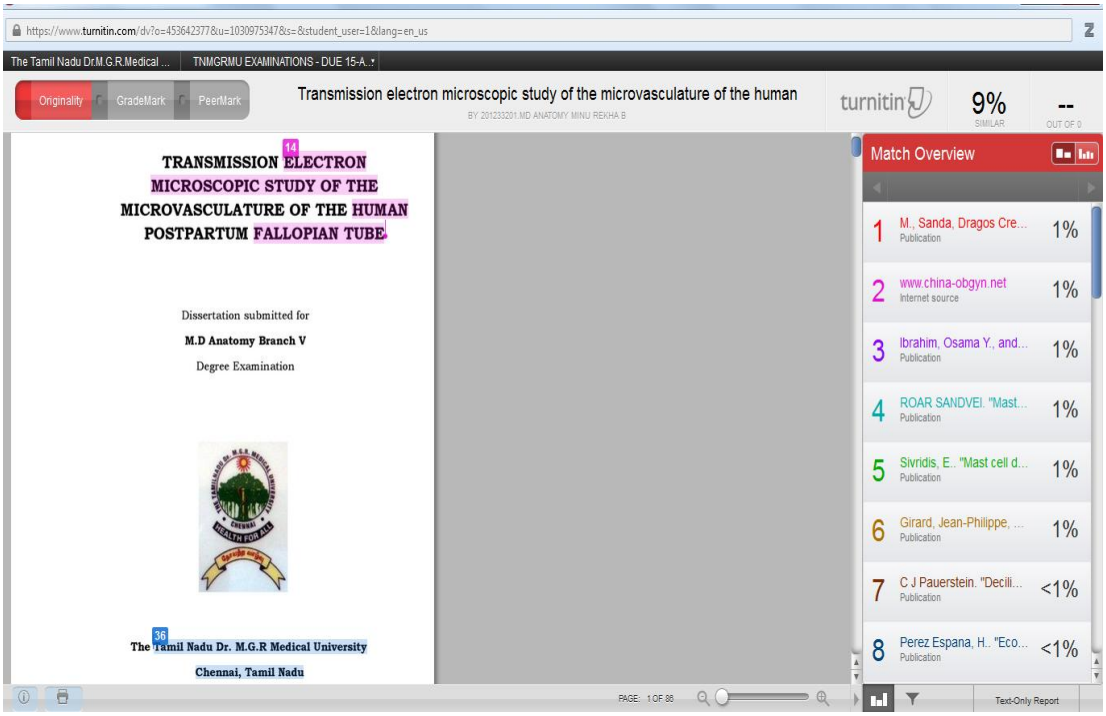
DECLARATION

I hereby declare that the dissertation entitled **"Transmission electron microscopic study of the microvasculature of the human postpartum Fallopian tube"** is a bona fied research work done by me under the supervision of Dr. Suganthy J., Professor of Anatomy, Christian Medical College, Vellore, in partial fulfilment of the requirements for the M.D Anatomy examination (Branch V) of The Tamil Nadu Dr. M.G.R Medical University, Chennai to be held in April 2015.

Name : Dr. Minu Rekha B.

M.D Anatomy (Branch V)

PLAGIARISM CERTIFICATE



ACKNOWLEDGEMENT

I would like to express my sincere thanks to each and every one individually, for without their support this thesis would not have taken its shape.

Dr. J. Suganthy, my guide for her constant support, valuable suggestions and encouragement throughout the study.

Dr. Bina Isaac, Head and professor, Department of Anatomy for her persistant support and help.

Dr. Santosh Joseph Benjamin, Assistant professor, my co-guide and Dr. Mapitha, senior resident, Department of Obstetrics and Gynaecology, for their enduring help in sample collection and also for the access to clinical data.

Dr. Visalakshi Jeyaseelan – Department of Biostaistics, for her availability and earnest guidance in the statistical analysis of my work.

Dr. Tripti Meriel Jacob and Dr. Samuel Frank Stephen, Department of Anatomy, for their valuable suggestions, support and help throughout my study.

Dr. Anna B Pullimood, Professor and Head, Mrs. Reeta Bascal and Mr. Sateesh, technicians, Central Electron microscopy

Facility, Wellcome laboratory, for technical assistance in electron microscopy.

All teaching and non – teaching staffs, Department of Anatomy, for all their timely help and co-operation throughout my study.

Mr. V. Gopinath, Secretary, Mrs. Amsaveni and Mrs. Akila Williams, technicians for their help in all technical aspects of the study.

Institutional Review Board, Christian Medical College, Vellore, for approving the study.

The Fluid Reseach Grant Committee, Christian Medical College, Vellore, for funding my work.

All the patients, who willingly participated in my study.

My beloved family members, for their prayers, support, care and constant encouragement.

Above all, I would like to thank the almighty God, for His blessings and being my strength throughout this work.

CONTENTS

ABSTRACT

TITLE OF THE ABSTRACT : Transmission electron microscopic study of the microvasculature of the human postpartum Fallopian tube.

DEPARTMENT : Anatomy

NAME OF THE CANDIDATE : Minu Rekha B.

DEGREE AND SUBJECT : MD Anatomy

NAME OF THE GUIDE : Dr. J. Suganthy, M.S., DNB, Ph.D.

OBJECTIVES OF THE STUDY:

To quantify the mucosal microvessels and intraepithelial lymphocytes (IELs) in the human postpartum Fallopian tube under light microscope and to look for the presence of high endothelial venules and their association with antigen presenting cells under transmission electron microscope.

METHODS:

The human postpartum Fallopian tube samples were processed for electron microscopy. Semithin sections, stained with 1% toluidine blue and ultrathin sections, stained with uranyl acetate and Reynold's lead citrate were obtained.

Semithin sections were examined under Olympus BX43 light microscope connected to a computer with cellSens image analysing software. Using

which, the number of mucosal microvessels and intraepithelial cells were quantified. Ultrathin sections were examined under transmission electron microscope (TECHNAI T12 SPIRIT) wherein, the microvessels were studied and mucosal mast cells quantified. Descriptive analysis, spearman's rho correlation and simple regression analysis of the data were obtained using SPSS version 17.0.

RESULTS:

The mean number of mucosal arterioles, capillaries, high endothelilal venules (HEVs) and venules per mm^2 were 29.23 $\pm$ 6.87, 161.38 $\pm$ 7.05, 19.23 $\pm$ 7.05 and 39.50 $\pm$ 9.96 respectively. The mean number of intraepithelial lymphocytes per 100 mm length of epithelium was 771.30 $\pm$ 300.85. There was a significant positive correlation between the number of IELs and HEVs ($p<0.001$) and IELs regressed on HEVs with the regression coefficient of 42.002. The mean number of mucosal mast cells per 1.5625 mm^2 was 40.30 $\pm$ 15.68. Migration of lymphocytes through mucosal HEVs and the association of antigen presenting cells (dendritic cells and macrophages) with microvessels were also observed. The presence of IELs, HEVs and the manner of interaction of IELs with the neighbouring epithelial cells proves that Fallopian tube is a member of MALT. It is hoped that these data from the study may provide some insights into the understanding of microvessels in the Fallopian tube under the influence of hormones during pregnancy and their association with immune cells.

Keywords: High endothelial venules, intraepithelial lymphocytes, mast cells, telocytes, clear cell variant.

1. INTRODUCTION

THE FALLOPIAN TUBES:

Gabriele Fallopio, one of the famous anatomists of the sixteenth century was the first to give the exact description of the Fallopian tubes and called them the 'trumpets of the uterus'. He considered that these tubes, extending from ovary to uterus, act as sperm conveying vessels (1). Fallopian tubes are otherwise named as oviducts, salphinges or uterine tubes.

ANATOMY OF FALLOPIAN TUBES:

Fallopian tubes are paired tubular structures that are attached to the upper part of the body of uterus. Its length ranges from 10 – 12cms. Each Fallopian tube opens at one end into the superior angle of the uterine cavity and at the other, into the peritoneal cavity. It consists of four parts, namely: (i) intramural, (ii) isthmus, (iii) ampulla and (iv) infundibulum with fimbriae (2).

TUBAL INNERVATION AND LYMPHATICS:

Fallopian tubes are innervated by autonomic nerves containing both sympathetic and parasympathetic nerve fibres (2–4). The parasympathetic preganglionic fibers are from pelvic splanchnic

nerve for the medial half and from vagus for the lateral half of the Fallopian tube (2,5). The preganglionic fibres of the sympathetic system are from T10 to L2 spinal segments (2). Lymphatics from the Fallopian tube drain into para-aortic nodes, internal iliac group of lymph nodes and can also reach the inguinal nodes via the round ligament (2).

HISTOLOGY OF FALLOPIAN TUBE:

Microscopically Fallopian tube has (i) mucosa (epithelium, subepithelial connective tissue and lamina propria), (ii) muscular layer and (iii) vascular serosa (2). Mucosa is highly folded and branched with narrow grooves separating them, thereby forming an irregular lumen. The epithelium of the Fallopian tube is simple columnar with both ciliated cells and non ciliated cells, namely secretory and peg cells lying on a prominent basement membrane (6). Cilia are about 10µm long and 0.25µm in diameter. The ciliated cells gradually decrease in proportion from the fimbrial end (50%) to the isthmus (less than 30%) (7). The secretory cells are columnar cells with microvilli. Peg cell are narrow cells with a dark stained nucleus and are considered to be secretory cells that have exhausted their secretions (8). Peg cells that are considered to be the variant of secretory cells, project into the lumen beyond the level of their neighbouring ciliated cells (2). The lamina propria of

the Fallopian tube is composed of loose connective tissue with abundant blood vessels (3). The muscular layer has inner thick circularly arranged and outer thin longitudinally arranged smooth muscle layers. The serosa consists of a thin layer of connective tissue lined by mesothelium (3,7). The epithelium of Fallopian tube is under the influence of oestrogen and progesterone. Oestrogen increases the number of ciliated cells and also the height of the cells. At the time of ovulation, the cells reach a maximum height of about 30μ. Progesterone, on the otherhand, increases the number of secretory cells (3,4).

Functions:

The Fallopian tube, besides serving as a mere conduit between the ovary and the uterus for the transport of gametes, is also the site of fertilization that occurs in the ampullary part, and transports the blastocyst into the uterus. This is effected by the epithelial ciliary activity, muscular contraction and composition of the tubal fluid (9). The secretory products of the epithelium also nourish the gametes and the blastocyst. It also serves to delay the transport of the embryo into the uterine cavity until it is well prepared for receiving the blastocyst for implantation (10) and has a role in sperm capacitation (11). The caudal part of isthmus in animals act as a reservoir for viable sperms until ovulation (12).

MUCOSA ASSOCIATED LYMPHATIC TISSUE (MALT):

The lymphatic aggregates located on the mucosal surface of tissues without a capsule is called mucosa associated lymphatic tissue (MALT). This diffuse lymphatic tissue is exposed to the external environment and provides an active defence against pathogenic organisms. They are the sites of innate immune response of both cellular and humoral varieties (4,13). Their presence in the gastrointestinal tract (gut associated lymphatic tissue - GALT) and respiratory tract – (bronchus associated lymphoid tissue – BALT) (4) and larynx (larynx associated lymphoid tissue – LALT) (14) are well known. Recently, their existence is also shown in conjunctiva (conjunctiva associated lymphoid tissue - CALT) (15) lacrimal glands (lacrimal duct associated lymphoid tissue - LDALT) (16) and salivary glands (duct associated lymphoid tissue - DALT). Cases of primary MALT lymphomas was also been reported in breast (17) and thyroid gland (18). In addition, the genitourinary system is also found to have aggregates of lymphoid tissue that are considered to be a member of MALT (4,19). The characteristic features of MALT include aggregation or diffuse arrangement of lymphocytes, other free immune cells like plasma cells and antigen presenting cells in the subepithelial layer of lamina propria (4). Most of the lymphocytes are B cells and among T cells, CD4+ helper T-cells predominate (20). It provides secretory immunity by

a) IgA secretion, b) immune response through T helper cells and c) immune tolerance through cytotoxic T cells (21).

MICROVASCULATURE:

Microvasculature or microvascular bed of a tissue is a part of the circulatory system which includes the small blood vessels namely arterioles, capillaries, postcapillary venules and venules (22).

(i) Arterioles:

Arterioles are described as arterial vessels with overall diameter less than 100µm. They are classified as arterioles, small arterioles, terminal arterioles (30µm-50µm) and meta-arterioles. The thickness of their walls seen in fixed section is only slightly less than the diameter of the lumen (8,23). The wall of an arteriole is composed of three layers from within outwards, namely tunica intima, media and adventitia. The tunica intima is lined by flattened endothelial cells with their basal lamina applied onto the fenestrated internal elastic lamina (8). Tunica media has circularly arranged smooth muscle cells limited by an external elastic lamina. The adventitia is as thick as the media in arterioles and is composed of collagen and elastic fibers (23–25). Arterioles are recognized based on the following features:

1) ***The wall thickness and the luminal diameter*:** as the arterioles branch, their wall and lumen becomes thinner but the ratio between their wall thickness and the luminal diameter remains unaltered
2) ***The number of layers of smooth muscle cells in the tunica media**:* typically arterioles have 1 – 3 layers of smooth muscle cells.

The internal and external elastic laminae become very thin in small arterioles and are absent in the smallest arterioles (23). The terminal arterioles are 30-50nm in diameter with a single continuous layer of smooth muscle cells. The meta-arterioles are wider than true capillaries with scattered smooth muscle cells in the first half of their course (8,23).

(ii) Capillaries:

Capillaries are the smallest blood vessels, usually of 8 – 10µm diameter, that connect the arterioles and venules. They enable exchange of oxygen, carbon-di-oxide, nutrients, water and many other waste products from tissues. They are lined by a layer of endothelium surrounded by a thin perivascular layer and are devoid of smooth muscle cells (23,25). The endothelial layer is made up of pavement like cells with centrally located oval nuclei cemented edge to edge with crenated margins, resting on a basement membrane. The perivascular layer is made of loose

connective tissue along with a discontinuous layer of pericapillary cells like histiocytes, fibroblasts and pericytes (23,25). There are three types of capillaries namely continuous, fenestrated and sinusoidal capillaries (2,26).

Under electron microscopy, the endothelial cells show an endocapillary layer made of glycoprotein lining their luminal surface. Some may show microvilli. Membrane bound electron dense granules (diameter 0.1 - 0.2µm) are present (25). They also show numerous pinocytotic vesicles. The open vesicles are called caveolae. The endothelial cells show tight cellular junctions which can be either end to end, overlapping or 'S' shaped (23,25). The pericytes are irregular shaped cells distributed at intervals along the capillaries. They are enclosed by the basal lamina of the endothelium. These cells are considered to be undifferentiated cells that can give rise to different types of cells including smooth muscle cells (24,25).

(iii) Venules:

Venules are small blood vessels in the microvascular bed that connect the capillaries to the veins. Their diameters range from 8 – 100 µm. They are classified as post capillary venules (8 – 30µm), collecting venules (30 - 50µm) and muscular venules (50 - 100µm) (8,24). The venules are lined by flattened endothelial cells lying on a basement membrane. The collecting venules have a thin

adventitia and a single layer of veil cells (primitive smooth muscle cells). The muscular venules, in addition, have two layers of smooth muscle cells and a thin ill-defined internal elastic lamina. All venules look alike under light microscopy except for the diameters (23,25). Ultra structurally, the endothelial cells of the venules lie over a 50nm thick basement membrane. There are gaps of 10-20nm instead of tight junctions (23,25). The endothelial cells are rich in actin containing microfilaments so that they can alter their shape and enable fluid and leucocytes to leave or enter the vessels. The adventitia is made of collagen fibres (25)

(iv) High endothelial venules (HEVs):

High endothelial venules are anatomically distinct post-capillary venules that are found in lymph nodes and secondary lymphoid tissues except for the spleen (27,28). Thome in 1898 was the first to describe these venules with a plump endothelial cells bulging into the lumen of the vessel. Endothelium of HEVs have a characteristic cuboidal morphology and a prominent Golgi complex, where unique sulfated glycans are synthesized (29).

High endothelial venules control the extravasation of naive lymphocytes from the blood into the afferent lymphatics (30). Gowans and Knight showed that radioactively labelled lymphocytes that were transfused into the blood of rats migrated rapidly into lymph nodes by HEVs. This experiment conclusively demonstrated

that HEVs are the site of large-scale migration of lymphocytes from the blood into lymph nodes (31). Naive T and B cells migrate through HEVs by the following steps, namely: rolling, sticking, crawling and transmigration (29).

Under light microscopy, HEVs are lined by cuboidal endothelium with the presence of infiltrating lymphocytes and a surrounding reticular sheath. An abrupt transition from flat to cuboidal cells can be seen where the capillaries join the HEVs and polygonal to flat endothelium seen at places where HEVs join the small veins (32).

Ultrastructurally, HEVs are lined by cuboidal endothelial cells (23,32). Their nuclei are large and lobular with loose chromatin condensed at the periphery, 10 - 12 nuclear pores and one or two prominent nucleoli (32).

The HEVs are surrounded by two to three layers of concentrically arranged reticular cell plates (32). The lymphocytes migrate radially across the successive laminations by insinuating themselves through gaps between the overlapping reticular plates (32).

The vascularity of the female reproductive system changes periodically under the influence of oestrogen and progesterone during each menstrual cycle (7) and pregnancy (33). Though many

studies on the microvasculature have been done in normal Fallopian tube and during early pregnancy in animal models, there is no information about this in the postpartum Fallopian tube. In addition, previous studies have shown that HEVs are absent in human Fallopian tube (34) yet an incidental finding in our department demonstrated HEVs in postpartum Fallopian tubes. In addition to lymphocytes, the HEVs act as a migratory route for plamocytoid dendritic cells (35), precursors of dendritic cells (36) and probably natural killer cells (37). The presence of HEVs, which are considered crucial for the immune cell trafficking, needs to be confirmed in the Fallopian tube. For the better understanding of the hormonal influence on the vasculature of Fallopian tubes, there is a need to estimate the microvasculature of the human postpartum Fallopian tube.

2. AIM AND OBJECTIVES

AIM:

To study the microvasculature of the human postpartum Fallopian tube under light and transmission electron microscope.

OBJECTIVES:

1. To quantify the number of mucosal arterioles, capillaries, high endothelial venules (HEV) and venules per unit area under light microscopy.

2. To quantify the number of intraepithelial lymphocytes per 100 millimetre length of the epithelium under light microscopy.

3. To look for the correlation between the number of intraepithelial lymphocytes with the number of mucosal HEVs.

4. To quantify the number of mast cells in the mucosa per unit area under transmission electron microscope (TEM).

5. To look for high endothelial venules and their association with lymphocytes and other antigen presenting cells under TEM.

3. LITERATURE REVIEW

FUNCTIONAL ANATOMY OF FALLOPIAN TUBE:

Fallopian tubes are paired tubular structures connecting the ovaries to uterus and are the sites of fertilization. Fallopian tubes play an important role in the transport of gametes and the zygote (38). The ovum released in the peritoneal cavity is captured by the fimbrial end of the Fallopian tube. The increased number of ciliated cells in the infundibulum beat towards the uterine cavity and transport it to the ampulla (3). Fertilization takes place in the lumen of the ampulla and the zygote formed is then transported to the uterine cavity (39,40). The non-ciliated peg cells are secretory in function and provide nutrition to the ovum and the fertilized zygote. The secretions also nourish the sperms and help in the process of capacitation (3).

Transportation of egg and zygote through Fallopian tube is facilitated by:

(i) muscular contraction under the influence of prostaglandin F alpha which in turn are influenced by the oestrogen and progesterone (41)

(ii) ciliary activity of the ciliated epithelial cells of the Fallopian tube (40,42)

(iii) tubal secretions (5)

(iv) microvascular architecture of the Fallopian tube (43).

These factors have been discussed in detail below:

(i) Muscular contraction

Smooth muscle contraction is produced in an oscillatory pattern in the isthmus which modulates the transport through the Fallopian tube. Stimulation of alpha adrenergic receptors promotes contraction of the oviductal muscle, although stimulation of beta receptors inhibits contractions (44). Adrenergic neurons may not be the primary means for controlling embryo transport since experimental depletion or inhibition of these neurons does not prevent transport nor decrease fertility (45). The sex steroid hormones and other factors produced by the oviduct itself, such as prostaglandins, nitric oxide and prostacyclin may also modulate muscle contraction and play a lead role in embryo transport (44,45).

(ii) Ciliary activity:

In addition to tubal smooth muscle contractility, tubal transport of embryo is influenced by ciliary activity. Factors such as sex steroid hormones and interleukin 6 (IL-6), found within the follicular fluid and released into the Fallopian tube during ovulation, have also shown to affect the ciliary activity of the

Fallopian tube epithelium. Although the relative importance of each of these mechanisms is not clearly understood, studies have shown that oocyte and blastocyst transportation within the Fallopian tube is mainly due to the ciliary activity of the epithelium (40,45). Studies have shown that when the tubal smooth muscle activity is inhibited by a β-adrenergic agonist like isoproterenol, there was no change in the time of transit of the embryo through the Fallopian tube. This proves that ciliary activity alone is sufficient to transport the embryo into the uterine cavity for implantation within the appropriate time period (45). Furthermore a Fallopian tube with a tubal ectopic pregnancy demonstrated a marked reduction in the number of ciliated cells in comparison with an intrauterine pregnancy of the same gestational stage. Marked de-ciliation is also sometimes seen subsequent to a tubal ectopic pregnancy and in biopsies from women undergoing tubal surgery, who later develop a tubal ectopic pregnancy (45).

(iii) Tubal secretions:

The Secretion of the tenacious mucus at the isthmus of the Fallopian tube during the oestrous phase of menstrual cycle, facilitate sperm passage into the tube. The disappearance of this mucus after ovulation facilitates the transport of embryo into the uterine cavity (5). The secretions of the secretory epithelium

contains an oviduct specific glycoprotein which has a crucial effect on the function of sperm, fertilization and the development of the zygote (46). The contents of the luminal fluid of the Fallopian tube have been found to harden the zona pellucida of the embryo thereby preventing polysomy. The proteins that play a role in hardening of zona pellucida are oviductal glycoprotein-1 (OVGP1) and heat shock protein (HSP) and protein disulphide isomerase (PDI) family members (47).

(iv) Microvasculature of the Fallopian tube:

The microvascular architecture of the Fallopian tube is also found to influence the transport of ova. Verco et al. demonstrated the existence of a sub-epithelial capillary plexus with high pressure blood in the isthmus and low pressure blood in the ampulla of rabbit Fallopian tube. They postulated that the net luminal secretion and absorption modified the luminal flow of fluid that resulted in the transport of ova from the fimbrial end to the uterine cavity (43). The presence of a sub-serosal venous plexus acting as a sphincter in the isthmic region, results in the delay of ova transport in the ampullo-isthmic junction (43) .

Derangement of any of these mechanisms may result in the improper transport of ova and zygote that may result in infertility or ectopic tubal pregnancy (45).

HORMONAL EFFECT ON FALLOPIAN TUBE EPITHELIUM:

(i) Changes of Fallopian tube epithelium during menstrual cycle:

Fallopian tube epithelium with both ciliated and secretory cells respond cyclically to estrogen and progesterone (48). Both the cell types are low cuboidal during the menstrual phase. During the proliferative phase the cells increase in height and reach their maximum height. Both cells attain equal height. During ovulation, the percentage of ciliated cells is more (49) and the secretory activity of the secretory cells is the maximum (50). The contents of the secretory cells are discharged into the lumen and therefore there is a relative decrease in the number of secretory cells (50). In addition, they fall short in height from the ciliated cells thereby facilitating the transport of the released ovum by the ciliary beat (51). In luteal phase both cell types decrease in size and partial deciliation is also seen. The epithelium also expresses estrogen and progesterone receptors. Estrogen receptors are predominant in midcycle and progesterone receptors are seen throughout the cycle. Estrogen stimulates the increase in cell size, secretion, and number of cilia, whereas progesterone causes cell atrophy and deciliation (50).

(ii) Changes of Fallopian tube epithelium during pregnancy:

A light and electron microscopic study on day two pregnant mice showed that the epithelium contains mainly secretory cells with microvilli and occasional ciliated cells (33).

(iii) Changes of Fallopian tube epithelium during puerperium:

In a study, human Fallopian tube specimens obtained on the 5th postpartum day were examined to evaluate puerperal changes in the tubal epithelium by scanning and transmission electron microscopy. Ciliated cells were most densely distributed on the fimbria and in the ampulla, and were relatively infrequent in the isthmus. There was a progressive decrease in the numbers of ciliated cells and deciliation of individual cells was also noted. The non-ciliated cells that were in resting stage at term pregnancy regained their secretory activity during the puerperium (52).

(iv) Changes of Fallopian tube epithelium during the perimenopausal and postmenopausal period:

Twenty biopsy specimens of human Fallopian tube were studied under TEM and Field emission scanning electron microscope (FE-SEM) to look for the changes in the luminal surface of the Fallopian tube epithelium in perimenopausal to postmenopausal women. It was observed that there was a gradual shortening of the microvilli and deciliation followed by development

of microplicae like structures. This transition was a slow gradual involutional process that happened over a period of 20 – 30 years after menopause probably accounting for the altered hormonal status (53). After menopause, the percentage of ciliated cells and the height of the epithelial cells are significantly decreased (49). Another study on eighteen women also confirmed that deciliation was found 20 - 30 years after menopause (54).

(v) Changes of Fallopian tube epithelium during hormonal therapy:

While estrogen influences ciliogenesis and increase in height of the epithelium, deciliation and decrease in height of cells was observed under progesterone therapy, these changes being easily reversible (49).

BLOOD SUPPLY OF FALLOPIAN TUBE:

The blood supply of Fallopian tube is derived from uterine and ovarian arteries. The lateral third is supplied by the ovarian artery and the medial two third by the uterine artery. The veins of the lateral two third drain into the IVC on the right, and the left renal vein on the left side via the pampiniform plexus. The medial one third drains in to the internal iliac veins (2).

Alterations in the blood flow to the uterus have been found to play an important role in reproductive physiology and also in a number of pathological disorders of uterus (55).

Verco et al. have studied the architecture of the microvessels of the isthmic, ampullary and fimbrial part of twelve virgin female rabbits using vascular corrosion cast with scanning electron microscopy. They noted that the presence of large veins in the plical core was one of the unique features of ampulla. Mucosal arterioles were infrequently identified. The sub-epithelial capillary density was greater. The fimbrial vascularity was also found to be more pronounced (58).

Their study further showed that the arteriolar supply was more in the isthmus and the venous drainage was more in the ampullary region. So in the ampulla, there was a net fluid absorption and in the isthmus there was a net fluid secretion and this current of fluid helped in the ovum pickup and also the gamete transportation (58).

HORMONAL INFLUENCE ON FALLOPIAN TUBE VASCULARITY:

The vascularity of the reproductive tract undergoes changes under the influence of the sex steroids oestrogen and progesterone. Progesterone is well known to increase the vascularity of reproductive organs. Vascular endothelial growth factor (VEGF)

within the Fallopian tube luminal epithelium induce angiogenesis and may increase vascular permeability, modulate tubal luminal secretions and plays an important role in the cyclical changes of vasculature in the Fallopian tube (57). VEGF, produced by the theca lutein cells of corpus luteum during early pregnancy is responsible for the angiogenesis of reproductive tract tissues (59,60). In a study, the endothelial cells did not show immune-reactivity for progesterone or oestrogen receptors in both uterus and Fallopian tubes, whereas immune-reactivity was seen in the muscle cells of uterine arteries. Thus sex steroid hormones may regulate uterine blood flow through a direct effect on uterine arterial walls. These receptors were also found in the arterial walls of the Fallopian tubes (56).

A study on day two pregnant mice showed that there were very few fenestrated capillaries in Fallopian tube and it was concluded that fenestrations were under hormonal influence, progesterone decreased the fenestrated vessels (33).

MUCOSA ASSOCIATED LYMPHATIC TISSUE IN FALLOPIAN TUBE:

The genital tract is considered to be a part of the common mucosal immune system. Fallopian tube, being a part of female genital

tract has lymphoid aggregations in the mucosa which are more abundant in the interstitial part (34). The functioning of such local immune system in the human Fallopian tube acts as a first line of defence against tubal infection and also prevents tubal factor infertility (61,62).

Intraepithelial lymphocytes (IELs):

Intraepithelial lymphocytes have been demonstrated in the Fallopian tubes (34,63,64). These cells show round to oval nuclei with peripherally condensed chromatin and electron lucent cytoplasm with few mitochondria and ribosomes. Complex interdigitations have also been observed between the cell membranes of the IELs with adjacent epithelial cells (61). It has been noted immunoelectron microscopically that the IELs are T cells and not B cells. The source of IELs is the follicles similar to that seen in GALT and BALT. They involve in immune tolerance, so that sperm and blastocyst transportation is possible without the trigger of local immune response (61).

Many studies have been done to estimate the frequency of IELs in the Fallopian tube during different phases of the menstrual cycle. Boehme and Donat studied 20 human Fallopian tube specimens and showed that there was no significant difference in the number of IELs between secretory and proliferative phases. The predominant types were CD3+ and CD8+ lymphocytes (65). Ulziibat

et al. demonstrated that the percentages of IEL per total number of epithelial cells were increased in the late proliferative and late secretory phases and in tubal pregnancies, whereas there were significant decreases in the proportion of IEL in the early secretory phase and post-menopausal specimens. They have also showed that none of the IELs were CD4+ or plasma cells and all the IELs were positive for estrogen receptor-β and not for estrogen receptor-α (66).

High endothelial venules in the Fallopian tube:

Lymphocytes continuously migrate from the blood stream to secondary lymphoid organs in search of their cognate antigens. Their circulation in the secondary lymphoid tissues or organs is achieved by their migration through lymph, followed by the blood stream and then back to the lymphoid tissues. Such a circulatory phenomenon is known as lymphocyte homing and is a crucial process for immune surveillance (67,68). The lymphocyte migratory process is mediated by sequential adhesive interactions between lymphocytes and specialized post-capillary venules, called high endothelial venules, in the secondary lymphoid organs (28,69–73). Otsuki et al. reported that there were no HEVs in the Fallopian tube and the lymphatic capillaries were said to be the migratory route of the lymphocytes (34).

MAST CELLS:

Mast cells are immune cells first described by Paul Ehrlich in his doctoral thesis in the year 1878 (74,75). They are seen in various organs and connective tissues. They appear in the tissues close to blood vessels or in tissues that are exposed to the environment like the gastrointestinal tract (GIT), respiratory tract and skin. Therefore, one of the functions of mast cells, is to defend against invasion by foreign agents (76).

The mast cell progenitors are derived from CD34+ haematopoietic stem cells in the bone marrow and do not mature until they leave the marrow. They then disperse in various tissues, complete their maturation and settle there (77). Based on their location, types of granules and the proteins they contain, they are classified into three types:

(i) Mast cell tryptase (MCT) – found in the mucosa of GIT and lungs

(ii) Mast cell tryptase-chymase (MCTC) – found in the connective tissue of skin, breast, heart, conjunctiva of eye and submucosa of stomach and intestine

(iii) Mast cell chymase (MCC) – found in the mucosa and sub-mucosa of stomach, small and large intestine. Of these MCT are immune associated and are found in association with T cells (76).

Stucture of mast cells:

Human mast cells are round to oval in shape with a diameter of about 8 - 20µm. They have round to oval nucleus with the cytoplasm having lots of secretory granules of diameter about 1.5µm. The secretory granules contain different types of proteins like tryptase, chymase, histamine, heparin, cytokines, urokinase, vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). These proteins allow the mast cells to migrate, cause smooth muscle contraction, change their environment, cause allergic symptoms, cause chemotaxis of immune cells, promote blood vessel growth, and repair the injured tissues (76,78).

Mast cells can be demonstrated using various staining methods that include Giemsa stain, toluidine blue, stains for sulfated acid mucopolysaccharides (AMP), Leder (chloracetate esterase) stain and immuno histochemistry for cell markers like CD2 , CD23, CD25, CD68, CD88, CD117, CD203c and for tryptase and chymase (76).

Functions of mast cells:

Mast cells contribute to immunological reactions by affecting lymphocyte growth, recruitment and function. Recent studies have shown that mast cells also play a crucial role in innate immunity (79).

Some of the diseases that involve mast cells are allergies, autoimmune diseases, interstitial cystitis, cardiomyopathy (80), atheroscelerosis (81), endometriosis (82) and mast cell related tumours like mastocytosis, mast cell leukemias and sarcomas (76). Tryptase, secreted by mast cells, has an inhibitory effect on sperm motility (83). It has been demonstrated that pregnancy rate has improved after treatment of infertile men with mast cell blockers like ketotifen (84–86).

Studies of mast cell in female reproductive organs reveal that in the uterus, there is no apparent difference in the number of mast cells between the normal proliferative and secretory phase endometria. Mast cells are seen exclusively in the basal layer of the endometrium and the relative paucity of mast cells in the functional layer may contribute to the immune tolerance of gestational endometrium to the implantation of the blastocyst (87). In intrauterine contraceptive device (IUCD) users, mast cells are found to be more in the muscularis externa than in the lamina propria. Moreover, in the muscularis externa, they are found predominantly related to smooth muscle fibres as opposed to the blood vessels. The increase in the mast cell number in IUCD users might be a factor in the pathogenesis of pelvic inflammatory disease and the ectopic pregnancies that occur in IUCD users (88).

ANTIGEN PRESENTING CELLS:

Antigen presenting cells are specialized cells that play an important role in the mucosal innate immunity. They have been demonstrated in the skin, mouth, lung, small intestine and genitourinary tract (89,90). Antigen presenting cells include dendritic cells, macrophages (89) and B lymphocytes (91). Dendritic cells are the 'professional' antigen presenting cells with a spider-like morphological appearance and their main function is to capture antigens, process them, migrate to lymph nodes, differentiate, mature and present the processed antigens to the T lymphocytes to initiate an immune response (89). Dendritic cells interdigitating between the epithelial cells of the mucosa are called Langerhans cells and those in the submucosa are called submucosal dendritic cells (92). The female genital tract is immunologically unique with a property of immune tolerance to sperms and blastocyst. Dendritic cells and macrophages play a crucial role in this. Constitutionally, Langerhans cells and submucosal dendritic cells are located in the epithelium and lamina propria respectively. During inflammation, monocyte derived dendritic cells are recruited from the peripheral blood (92). Dendritic cells and macrophages are also under the influence of sex hormones (87).

During pregnancy, strict regulation of innate and adaptive immunity at the maternal–fetal interface is needed for the survival of the embryo. Simultaneously, effective immunity must be maintained in mucosa to protect both mother and foetus from harmful pathogens. Dendritic cells play an important role in immune tolerance and immune surveillance (93).

TELOCYTES:

A new cell type which was initially called interstitial Cajal like cells (ICLCs), were named "telocytes" by Popescu, as they differ in their electron microscopic, immunochemical and cell culture behaviour (94). Electron microscopically, telocytes are identified by the presence of their long, thin cytoplasmic processes termed as telopodes. Telocytes might contain 1 – 5 telopodes of length ranging from ten to hundreds of microns. The telopodes are made of alternating thin and dialated segments which are called podomeres and podoms respectively. The podoms contain numerous caveolae, mitochondria and rough endoplasmic retinacula. The thicknesss of telopodes are usually less than 0.2µm (95,96). According to the number telopodes, telocytes are classified as 1) piriform (one telopode) 2) spindle (two telopodes), 3) triangular (three telopodes) and 4) stellate (more than three telopodes)(94). Immunohistochemically, telocytes are c-Kit/CD117

positive and co-express CD34+ (95), connexion 43. However, their negativity for CD1a and CD62P help to differentiate them from other immune cells (94). They can also be identified using vital methylene blue stain based on their typical stucture (97).

Telocytes in female reproductive organs:

(i) In uterus:

Telocytes have been isolated from the uterus and Fallopian tube wall, cultured and studied for the presence of estrogen and progesterone receptors using monoclonal antibodies (98). These cells were c-kit positive and CD117 positive. Most of the CD117 positive cells co-expressed CD34 and vimentin. The telocytes in the uterus were found to be positive for both estrogen receptor α and progesterone receptor (98). In the uterine myometrium, telocytes establish relationships with capillaries, nerve fibers and with other interstitial cells like macrophages, mast cells, lymphocytes and eosinophils. They are interconnected with each other and with smooth muscle cells through cell-to-cell point contacts or gap junctions (98).

(ii) In Fallopian tube:

Telocytes in Fallopian tube are predominantly seen in lamina propria and in between smooth muscle fibres. Telocyte spatial distribution gradient decreases from the sub-epithelial area to the

serosa (98). They are associated with nerve endings but their association with blood vessels and immune cells have not been established in Fallopian tube unlike the telocytes seen in uterus (98).

Functions of telocytes:

1. Telocytes are proposed to act as stretch receptors if located near smooth muscle fibers in Fallopian tubes and uterus (95).
2. It has also been suggested that telocytes could themselves be stem cells (99), playing a part in muscle regeneration (100).
3. Telocytes have also been found to establish contact with the immune cells like plasma cells and eosinophils (98).
4. The presence of steroid hormone receptors in telocytes could behave as sensors, controlling the Fallopian tube peristalsis by signaling mechanisms depending on ovarian hormone levels (accelerated by estrogens and delayed by progesterone) (98).

4. MATERIAL AND METHODS

This study was approved by the Institutional Review Board, Christian Medical College (CMC), Vellore, India (IRB Min. No. 8314 dated 18.06.2013).

STUDY SUBJECTS:

Pregnant women at term admitted for lower segment caesarean section with sterilization in the Department of Obstetrics and Gynaecology, CMC, Vellore from August 2013 to April 2014 were recruited into the study.

INCLUSION CRITERIA:

This study included ten pregnant women, who underwent lower segment caesarean section with sterilization by modified Pomeroy's technique.

EXCLUSION CRITERIA:

1. Previous history of pelvic inflammatory disease
2. Immunocompromised patients
3. Twins or multiple pregnancy
4. Patients unwilling for the study

SAMPLE SIZE ESTIMATION:

In the absence of earlier data in this field, pilot measurements were conducted, wherein measurements of the mucosal arterioles, capillaries, high endothelial venules and venules of two postpartum Fallopian tubes were quantified under light microscopy. These values were used to calculate the sample size for this study. The required sample size to quantify the microvessels was calculated with the formula,

$$N = \frac{Z_\alpha^2 \times sd^2}{d^2}$$

N - number of samples

Z_α - Z value of α error

Sd - standard deviation

d - precision

The sample size was found to be ten with a standard deviation of 2.3, 95% confidence limits, 1.5 units precision, with an alpha error of 5% and power of 80%.

COLLECTION OF SAMPLES:

After getting informed consent from ten patients, who underwent lower segment caesarean section with sterilization in the Department of Obstetrics and Gynaecology, Christian Medical

College, Vellore, the ampullary part of both the Fallopian tubes were obtained. The age of the patients ranged from 21 – 39 years. The fresh specimens were collected from the theatre in the 3% gluteraldehyde (EM fixative) solution in labelled airtight glass bottles, transported to the Department of Anatomy, CMC, surrounded in ice and were processed for electron microscopy.

PROCESSING FOR ELECTRON MICROSCOPY:

Chemicals used for tissue processing:

(I) PRIMARY FIXATIVE:

3% Gluteraldehyde in 0.1M sodium cacodylate buffer (pH 7.4)

Solution A – 25% Gluteraldehyde – 10 ml

Sodium cacodylate – 1.78 gm

Millipore water - 50 ml

Solution B – Calcium chloride – 21 mg

Millipore water – 10 ml

Solution A and B were mixed and pH adjusted to 7.4 using 1N sodium hydroxide.

The volume was made to 83.3ml using Millipore water, the solution filtered and stored at 4°C.

(II) WASH BUFFER:

0.1M sodium cacodylate buffer (pH 7.4)

Sodium cacodylate – 2.14 gm

Calcium chloride – 73 mg

Sucrose – 4 gm

Millipore water – 100 ml

The above chemicals were mixed and the pH adjusted to 7.4 using 1N hydrochloric acid. The solution was filtered and stored at 4°C.

(III) SECONDARY FIXATIVE:

Stock solution – 2% osmium tetraoxide

1gm ampoule of osmium tetraoxide was taken. After removing the label, the ampoule was washed with soap and water and rinsed with Millipore water and dried. A score on the ampoule was marked by a glass marker. The ampoule was dropped into a clean, dry, stout, brown coloured, glass bottle with stopper. Twenty five millilitres of Millipore water was added and closed so that it was airtight. The bottle was shaken until the ampoule broke. To this 25ml of Millipore water was added and shaken periodically for 24 hours. A double container was used to store this solution at 4°C in dark place.

0.2M sodium cacodylate buffer (pH 7.4) – (for preparation of 1% osmium tetraoxide)

Sodium cacodylate – 4.28 gm

Calcium chloride – 73 mg

Sucrose – 4 gm

Millipore water – 100 ml

All the above chemicals were mixed and the pH adjusted to 7.4 using 1N hydrochloric acid. The solution filtered and stored at 4°C.

Working solution – 1% osmium tetraoxide

Just before processing, the working solution was prepared freshly by mixing equal volumes of stock solution and 0.2M sodium cacodylate buffer. These were mixed well and added to the tissue for secondary fixation.

(IV) GRADED SOLUTIONS OF ETHANOL:

(i) 50% ethanol

(ii) 70% ethanol

(iii)95% ethanol

(iv)100% ethanol

The alcohol was diluted using Millipore water and stored at room temperature (20°C).

(V) RESIN MIXURE PREPARATION:

TAAB resin embedding kit (E008) was stored at 4°C.

The resin was thawed before preparing the mixture. Resin was prepared freshly for each use. For preparing a resin mixture of volume 20 ml, the following chemicals were used:

Araldite CY212 resin	- 10ml in a plastic syringe
DDSA (hardner)	- 10ml in a plastic syringe
Dibutyl phthalate (plastisizer)	- 4 drops in a glass pipette
DMP 30 (accelerator)	- 0.2ml in a plastic syringe

The above chemicals were added successively in the given order in a glass beaker. The mouth of the beaker was covered using a parafilm, mixed well using a magnetic stirrer for 30 minutes and allowed to stand at room temperature until the bubbles settled down. Excess mixture was stored at -25°C in airtight vials with the rubber stopper covered using parafilm.

(VI) CLEARING AGENT OR LINK SOLUTION:

Propylene oxide (TAAB laboratories)

(i) Propylene oxide

(ii) Propylene oxide + resin mixture is prepared by mixing equal volume of them at room temperature.

(VII) STAINING SOLUTIONS:

(i) 1% toluidine blue in 1% borax – for semi-thin Sectioning

Stock solution:

100 ml of Millipore water was taken in a glass beaker and 1 gm of sodium tetraborate was added to it and stirred well. To this, 1 gm of toluidine blue was added and stirred until it mixed completely, filtered and stored in an airtight bottle at room temperature.

Working solution:

Millipore water was added to the stock solution in the ratio of 3:1 and the solution was prewarmed on a hotplate at 60°C.

(ii) 2% uranyl acetate:

Saturated aqueous solution was prepared by adding 0.5 gm of uranyl acetate to 10ml of Millipore water, stirred well for 5 minutes

and centrifuged. The mid-portion of the centrifugate was taken and filtered through 0.2 Millipore filter and stored at 4°C in a plastic syringe with a Millipore filter attached to its hub.

(iii) Reynolds lead citrate:

Lead nitrate – 1.33gm

Sodium citrate – 1.76 gm

30ml of Millipore water was taken in a 50 ml volumetric flask. The chemical mentioned above were added to it, shaken for 1 minute and left in a magnetic stirrer for 30 minutes. Once a white precipitate of lead was formed 8ml of 1N sodium hydroxide was added gradually and stirring continued. 50ml of solution was made by adding Millipore water to it. It was aliquot in 10 ml disposable syringes and the air spells were removed as carbon-di-oxide would form a precipitate with lead citrate. This solution was stored in the plastic syringes with a Millipore filters attached to their hubs at 4°C.

At the time of staining, the required amount of solution was centrifuged, filtered through 0.2 Millipore filter paper and loaded in a plastic syringe without air bubbles.

Procedure for Tissue processing:

(I) PRIMARY FIXATION:

The obtained samples were trimmed and cut into pieces approximately 1mm dimension using new razor blade. Tissues were then fixed in 3% gluteraldehyde with 0.1M sodium cacodylate buffer (pH 7.4) for 5 days at 4°C in labelled, glass bottles. Buffer wash was given with 0.1M sodium cacodylate solution, 3 changes each, for 30 minutes, at 4°C.

(II) SECONDARY FIXATION:

Tissues were then fixed secondarily with 1% osmium tetraoxide in a dark room with agitation at 4°C for 3 hours. This was followed by 2 changes of buffer wash for 10 minutes each, at 4°C.

(III) DEHYDRATION:

Fixed tissues were then dehydrated with increasing grades of ethanol at room temperature, as follows:

50% ethanol – 15 minutes

80% ethanol – 15 minutes

95% ethanol – 15 minutes

100% ethanol – 15 minutes

100% ethanol – 15 minutes

(IV) CLEARING:

Tissues were then treated with two changes of propylene oxide followed by a mixture of propylene oxide with the resin. Clearing was done at room temperature.

Propylene oxide I – 15 minutes

Propylene oxide II – 15 minutes

Propylene oxide + Resin (1:1) – 30 minutes

(V) RESIN INFILTRATION:

The tissues were infiltrated with 3 changes of resin mixture (Araldite CY212) in clean glass vials with rubber stoppers for the following time durations at room temperature:

Resin I – 2 hours

Resin II – overnight

Resin III – 24 hours

(VI) EMBEDDDING AND CURING:

The silicone moulds were prewarmed in the incubator. The tissue pieces were then embedded in the prewarmed silicone mould with the cutting surfaces properly oriented, labelled and kept in the incubator for hardening at 60°C for 48 hours. The blocks were retrieved from the mould and stored in air tight plastic containers until sectioning.

Procedure for sectioning and staining:

(I) SEMITHIN SECTIONING AND STAINING

Semithin sections were taken from four blocks for each sample with a total of 40 blocks. The blocks were trimmed and 1µm semithin sections were taken using Ultrotome – Nova microtome with a glass knife made with a glass knife cutting machine. The glass knives thus made were fitted with a trough and the trough was filled with Millipore water during sectioning.

From each block, ten to twelve sections obtained were allowed to float on water in the glass knife trough. Of these 5 - 7 sections were selected randomly and placed on a water drop on a precleaned, dry glass slide using a tooth pick. The slides were kept on the hot plate at a temperature of 60°C and allowed to dry so that water gets evaporated and the sections were fixed to the slides.

The dried prewarmed slides were then stained with 1% toluidine blue in 1% borax solution mixed with Millipore water in the ratio of 1:3 for 1 minute at 60°C. The slides were then washed in tap water allowed to dry, cleared by dipping in xylene and then mounted with a cover slip using Distrene 80, dibutyl phthalate xylene (DPX).

(III) ULTRATHIN SECTIONING AND STAINING:

After the slides were studied under light microscope, two blocks from each sample were selected and the areas of interest in the semithin sections were marked for ultrathin sectioning. The area for sectioning was first trimmed with a new clean razor blade washed in ethanol. The ultrathin sections were cut using an ultra microtome (Leica Ultracut UC7) with a diamond knife (DIATOME) and the sections were allowed to float in the trough filled with Millipore water fitted to the diamond knife.

The sections of thickness 100nm were selected based on the golden-yellow colour of the sections when viewed under microscope. The sections were then allowed to flatten by applying xylene vapours using a brush dipped in xylene. The selected flattened sections were then mounted on the washed and precleaned 200 mesh copper grids, dull side up, using an eyelash stuck to a toothpick. The grids with the sections were then placed gently on labelled filter paper kept covered in a petridish. The sections were allowed to dry and stored.

The copper grids containing the sections were lifted carefully using tweezers and were stained by placing them dull side down on droplets of freshly prepared solution of uranyl acetate on parafilm kept in a petridish, along with moist filter paper, for 2 hours. The petridish was closed in order to prevent light exposure during

staining. After 2 hours, the copper grids were lifted using tweezers and gently washed in 3 changes of Millipore water in glass beakers. Each change involved 5 – 10 dips in the Millipore water.

Copper grids were counter-stained by placing them dull side down over droplets of the Reynolds lead citrate on parafilm kept in a petridish along with moist filter paper and pellets of sodium hydroxide for exactly 6 minutes. The grids were then washed 3 changes of Millipore water with 15 to 20 dips for each change. The grids were then gently placed on the labelled petridish using tweezers and allowed to dry for one day before examining under transmission electron microscope (TECNAI T12 SPIRIT) at 60KV.

QUANTIFICATION OF MUCOSAL MICROVESSELS AND INTRA EPITHELIAL CELLS BY LIGHT MICROSCOPY:

The stained slides were examined under light microscope Olympus BX43 fitted with Olympus DP21 camera connected to a computer installed with cellSens image analysing software (version 1.4)

1. The number of mucosal arterioles, capillaries, high endothelial venules and venules were counted under 40X magnification using the closed polygon measuring option of the image analysing software in 20 fields, each field measuring

approximately 50,000μm^2 (Figure 4.1). Thus, the above vessels were counted for a total area of 1mm^2 for each block.

2. The number of intraepithelial lymphocytes were counted under 40X magnification using the polyline measuring option of the image analysing software. The length measuring line was drawn along the basement membrane of the epithelium (Figure 4.2). A total length of 25mm was measured per block.

3. Areas of interest in the mucosa including the arterioles, capillaries, HEVs and venules were photographed under 4X, 10X, 20X, 40X and 100X (oil immersion) magnification using Olympus DP21 camera.

Statistical analysis of light microscopic data:

The data was statistically analysed using the SPSS version 17.0.

1. The descriptive statistics, namely, the minimum, maximum, mean, standard deviation, median and inter-quartile intervals were obtained for the variables counted and measured.
2. Spearman's rho correlation test was done to look for correlation between the number of mucosal HEVs and IELs.

3. Simple regression analysis was done between HEVs and IELs and the regression coefficient was calculated. The regression between IELs (the dependent variable) and HEVs (the independent variable) was sought.
4. Spearman's rho correlation test was done to look for correlation between age and the number of microvessels and IELs.
5. Spearman's rho correlation test was done to look for correlation between the parity and number of microvessels and IELs.

QUANTIFICATION OF MUCOSAL MAST CELLS AND THE STUDY OF THE HEV ASSOCIATION WITH APCS UNDER TEM:

The dried sections on the copper grids were examined under transmission electron microscope (TECNAI T12 SPIRIT) at 60KV.

1. For each sample, the number of mast cells in hundred squares of the copper grid was counted. Each square enclosed an area of 15,625 μm^2. Therefore the number of mast cells per sample was counted in a total area of 1.5625mm^2. (Figure 4.3)
2. In each section, the arterioles, venules, capillaries and HEVs of interest were photographed.
3. Antigen presenting cells and their relation with blood vessels were studied.

4. HEVs with associated lymphocytes and granulocytes were photographed.

5. Telocytes and their relation to blood vessels were looked for and were photographed.

Statistical analysis of TEM data:

The data were statistically analysed using the SPSS version 17.0.

1. The descriptive statistics, namely, minimum, maximum, mean, standard deviation, median and inter-quartile intervals were obtained for the mast cells in the mucosa of the Fallopian tube.

2. Spearman's rho correlation test was done to look for correlation between the age, parity and number of mast cells.

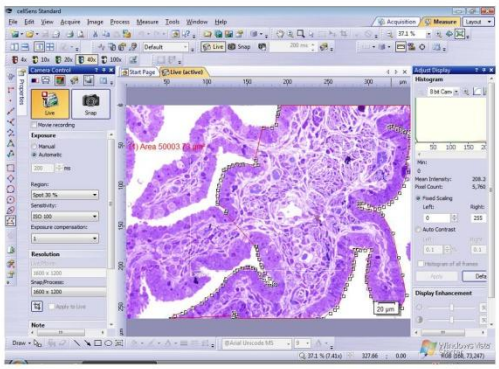


Figure 4.1: The area marked by the dotted line represents 50,000µm^2 of the human postpartum Fallopian tube mucosa using cellSens image analysing software. Microvessels in 20 such areas were counted in each sample.

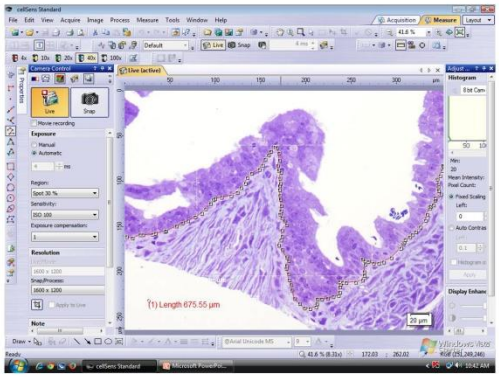


Figure 4.2: The dotted line represents the basement membrane of the human postpartum Fallopian tube epithelium. The number of intraepithelial lymphocytes was counted in a total length of 100mm in each sample using cellSens image analysing software.

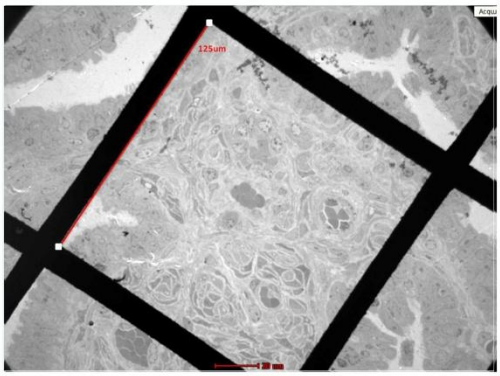


Figure 4.3: A single square of a copper grid is shown here. The number of mast cells in the mucosa of human postpartum Fallopian tube was counted in 100 such squares.

5. RESULTS:

The microvessels of the ampullary part of the human postpartum Fallopian tube were studied in females of mean age group 29.45 ± 6.53 and parity ranging from 2 – 4.

LIGHT MICROSCOPY:

The mucosa of the ampullary portion of the human postpartum Fallopian tube was highly branched and folded. The core of the mucosal folds contained lamina propria which was composed of fibroblasts in a reticular network of collagen and reticular fibers. But most of the folds also contained a longitudinally arranged smooth muscle layer within their core.

Epithelium:

The mucosal epithelium consisted of simple columnar epithelium with ciliated cells, secretory cells and peg cells lying on a wavy basement membrane (Figure 5.1). In some places the epithelium was observed to be stratified (Figure 5.2). The ciliated cells were pale stained, with numerous cilia on their apical surfaces which protruded into the lumen. Their cytoplasm showed numerous granules and a large, round, euchromatic nucleus, usually with a single, prominent nucleolus (Figure 5.3). In contrast, the secretory cells (Figure 5.3) were darkly stained, narrow columnar cells with a darkly stained, vertically oriented, oval nucleus with 1 - 2

prominent nucleoli (Figure 5.3). The peg cells were small cells with very little cytoplasm usually seen intercalated between the adjacent ciliated and secretory cells. The apical portion of these cells bulged into the lumen (Figure 5.3). The epithelium also showed numerous intra epithelial lymphocytes (Figure 5.1 & 5.2). In addition to these cells, a clear cell variant of the ciliated cells was also observed. They had very pale, clear cytoplasm with a large, euchromatic nucleus located at the centre of the cell, with prominent nucleoli and cilia on their apical surfaces (Figure 5.4).

In the mucosal folds, arteries and veins were present in addition to the microvessels. The muscular layer showed inner circular and outer longitudinal smooth muscle layers but an innermost spiral or longitudinal smooth muscle coat was also observed in most of the samples (Figure 5.5). Numerous mast cells were seen in both mucosal and muscular layers (Figure 5.6).

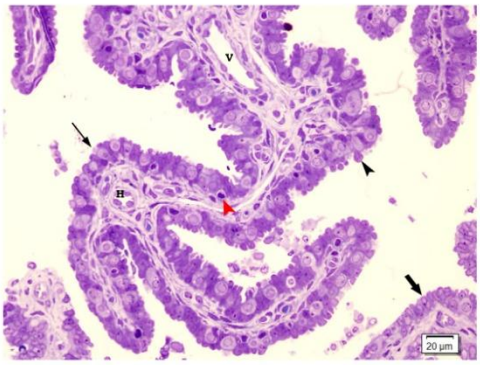


Figure 5.1: A Toluidine blue stained semithin section of a mucosal fold of human postpartum Fallopian tube showing simple columnar epithelium with ciliated cells (thin arrow), secretory cells (thick arrow) and peg cells (black arrow head). Intraepithelial lymphocytes (red arrow head) are also seen dispersed -within the epithelium.

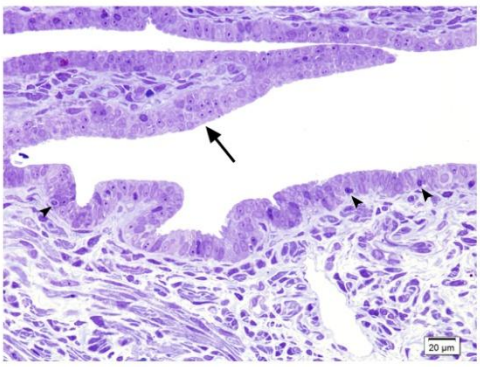


Figure 5.2: A mucosal fold of the human postpartum Fallopian tube showing stratified epithelium (arrow). Singly arranged round, oval and polygonal shaped intraepithelial lymphocytes are seen (arrow heads).

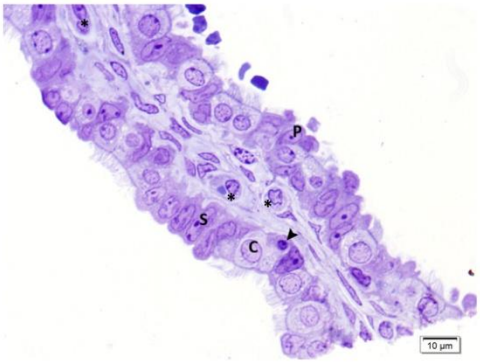


Figure 5.3: A mucosal fold of the human postpartum Fallopian tube showing pale stained, ciliated columnar cells (C), dark stained, secretory cells (S), peg cells (P) and an intraepithelial lymphocyte (arrow head) within the epithelium. Lamina propria shows blood vessels (*).

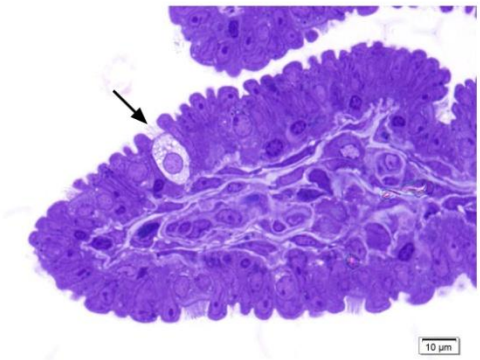


Figure 5.4: A toluidine blue stained section of a mucosal fold of the human postpartum Fallopian tube showing a clear cell variant of ciliated cell (arrow) in the epithelium.

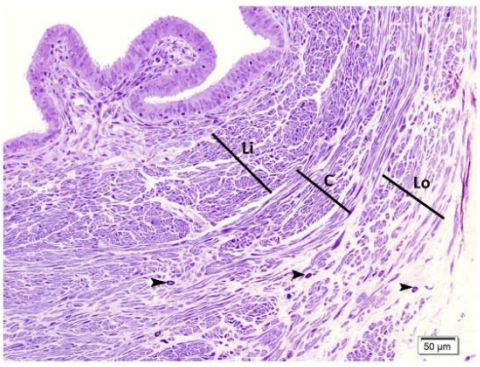


Figure 5.5: A Section of the human postpartum Fallopian tube showing the mucosal and muscular layers. The muscular layer shows inner longitudinal (Li), middle circular (C) and outer longitudinal (Lo) smooth muscle layers. Numerous mast cells are also seen in the muscular layers (arrow heads).

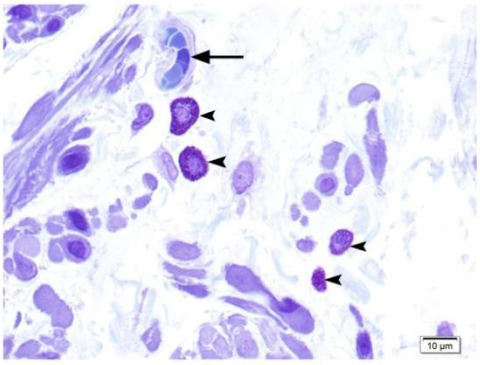


Figure 5.6: A toluidine blue stained section of the human postpartum Fallopian tube showing numerous mast cells (arrow heads) in the muscular layer. A mast cell is seen located close to a blood vessel (arrow).

Micro vessels:

Under light microscopy, the microvessels were found to be abundant in the lamina propria of the human postpartum Fallopian tube (Figure 5.1).

Arterioles:

The arterioles were seen predominantly in the basal and intermediate portions of the mucosal folds. They showed a tunica intima with endothelium, a tunica media with a single layer of smooth muscle cells and a thin tunica adventitia externally (Figure 5.7). Their lumina were regular and contained blood cells. The mean number of arterioles per square millimetre area was found to be 29.23 $\pm$ 6.87 (Table 1, Chart 1).

Capillaries:

Capillaries were the most abundant vessels found. The mean number of capillaries per square millimetre area of the mucosa was found to be 161.38 $\pm$ 36.86 (Chart 1, Table 1). The capillaries were most often found just beneath the basement membrane of the epithelium (sub-epithelial). They were all of the continuous type, lined by flattened endothelial cells. Erythrocytes, lymphocytes and granulocytes were also seen within the lumen of the capillaries.

High endothelial venules:

High endothelial venules, which are considered to be the migratory routes of lymphocytes in the mucosa associated lymphoid tissue of the body, were also seen in the mucosa of the human postpartum Fallopian tube. They were seen predominantly in the apical and intermediate portions of the mucosal folds and also close to the basement membrane of the epithelium similar to the capillaries (Figure 5.1). They showed a single layer of endothelial cells that were cuboidal in nature. The endothelial cells appeared to protrude into the lumen. The endothelial lining was surrounded by a thin layer of adventitia. The tunica media with smooth cells was absent in these vessels. Pericytes were also seen in these vessels. Their lumina showed the presence of erythrocytes and lymphocytes predominantly. But neutrophils were also found within the lumen of the HEVs (Figure 5.8). The mean number of the mucosal high endothelial venules per square millimetre area was 19.23 $\pm$ 7.05 (Table 1, chart 1).

Venules:

The venules were also found more towards the basal and intermediate portions of the mucosal folds, similar to the arterioles. However they had irregular and dilated lumina in contrast to the

arterioles. Their lumina also showed presence of various blood cells (Figure 5.1 & 5.7). The venules were found to be more in number compared with the number of arterioles. The mean number of venules per square millimetre area was found to be 39.50 $\pm$ 9.96 (Table 1, Chart 1).

Table 1: Microvessel frequency (mean per 1 mm^2 mucosal area) of the human postpartum Fallopian tube studied under light microscope.

S. no	Name of the microvessels studied	Mean number of vessels per mm^2 area of the mucosa	SD	Range		Median (IQR)
				Min	Max	
1	Arterioles	29.23	6.87	18.25	38.00	30.00 (23.38 -35.63)
2	Capillaries	161.38	36.86	103.25	223.00	167.50 (136.38 – 189.69)
3	High endothelial venules	19.23	7.05	8.50	31.50	18.50 (13.25 –24.31)
4	Venules	39.50	9.96	23.25	56.75	40.38 (33.63 – 45.25)

SD – Standard deviation, Min – minimum, Max - maximum IQR = Interquartile range (25 percentile and 75 percentile).

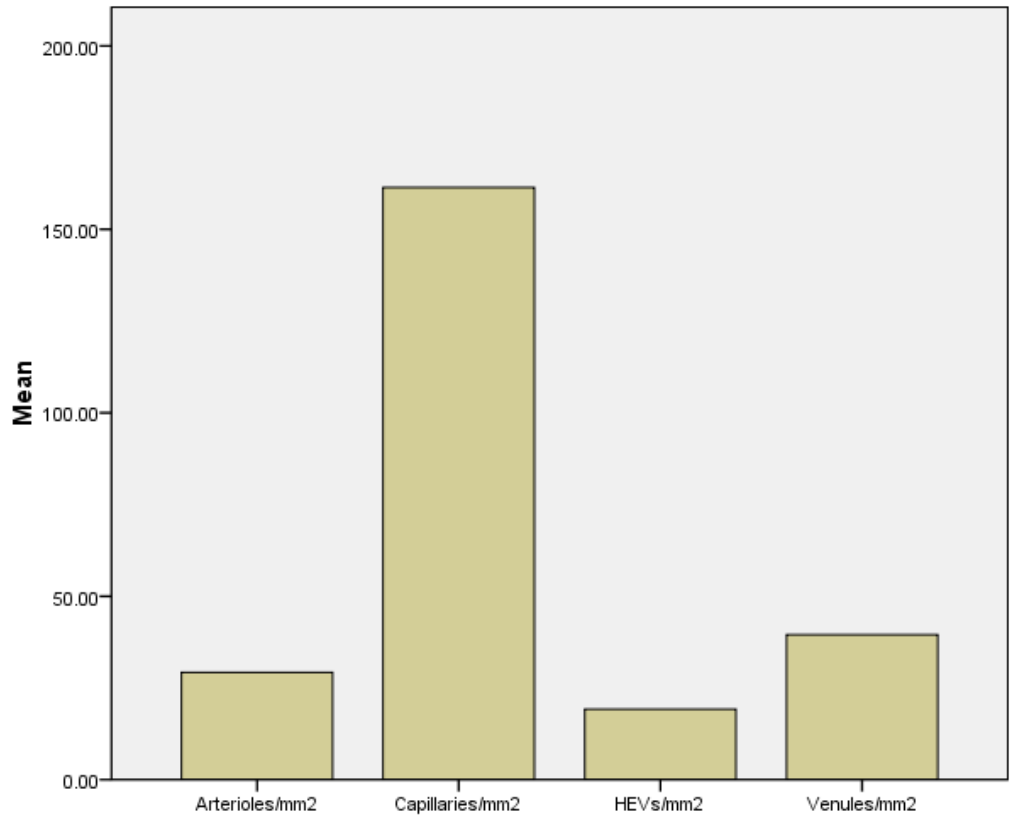


Chart 1: Bar chart showing the mean number of micro vessels per mm^2 area of the mucosa of human postpartum Fallopian tube studied under light microscope.

The mean numbers of the four categories of microvessels studies were compared as pairs by one way ANOVA followed by post hoc (Scheffe) test. Statistically significant differences ($p < 0.001$) were detected between the mean number capillaries and arterioles, capillaries and HEVs, and capillaries and venules (Table 2).

Table 2: Multiple comparison of the mean number of microvessels in the human postpartum Fallopian tube using one way ANOVA followed by Scheffe test.

(I) grp	(J) grp	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	Capillaries	-528.60000*	35.27009	.000	-632.0250	-425.1750
Arterioles	HEVs	40.00000	35.27009	.734	-63.4250	143.4250
	Venules	-41.10000	35.27009	.717	-144.5250	62.3250
	Arterioles	528.60000*	35.27009	.000	425.1750	632.0250
Capillaries	HEVs	568.60000*	35.27009	.000	465.1750	672.0250
	Venules	487.50000*	35.27009	.000	384.0750	590.9250
	Arterioles	-40.00000	35.27009	.734	-143.4250	63.4250
HEVs	Capillaries	-568.60000*	35.27009	.000	-672.0250	-465.1750
	Venules	-81.10000	35.27009	.172	-184.5250	22.3250
	Arterioles	41.10000	35.27009	.717	-62.3250	144.5250
Venules	Capillaries	-487.50000*	35.27009	.000	-590.9250	-384.0750
	HEVs	81.10000	35.27009	.172	-22.3250	184.5250

*. The mean difference is significant at the 0.05 level.

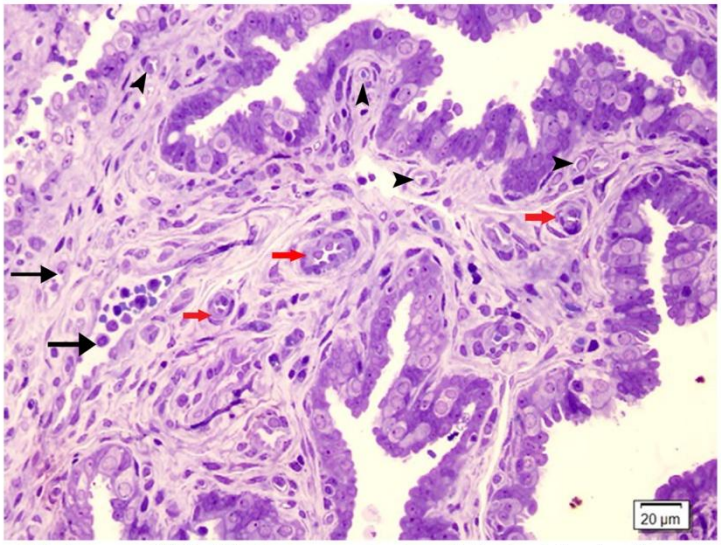


Figure 5.7: A mucosal fold of the human postpartum Fallopian tube showing numerous microvessels in the lamina propria. Arterioles (red arrrows) and venules (black arrows) are in the basal and intermediate portions of the fold whereas capillaries (arrow heads) are subepithelial in location.

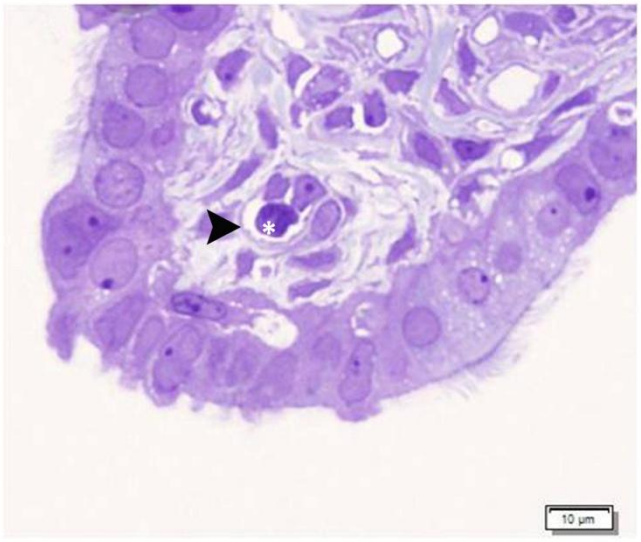


Figure 5.8: The apical portion of a mucosal fold of the human postpartum Fallopian tube showing a subepithelially located high endothelial venule (arrow head) with a neutrophil (*) within its lumen.

Arterioles showed a statistically significant increase with parity ($p < 0.05$), whereas the capillaries ($p < 0.05$) and HEVs ($p < 0.05$) showed a statistically significant decrease with parity (Chart 2, 3, 4). Venules showed increasing trend with parity but was not statistically significant (Chart 5). No significant correlation was found between age and microvessels.

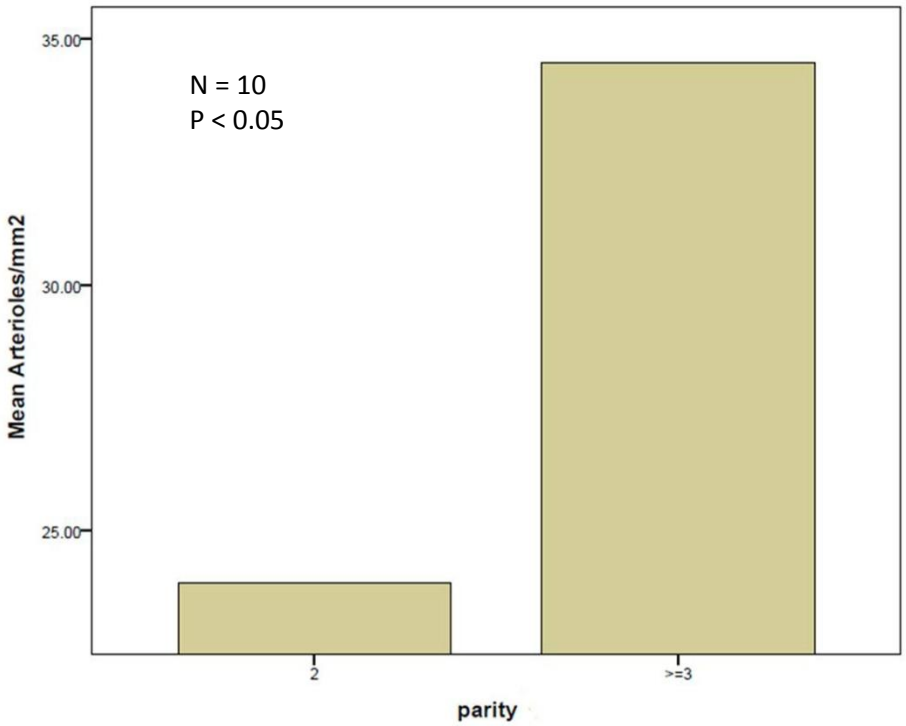


Chart 2: Bar chart of mean number of arterioles per mm^2 area of mucosa of human postpartum Fallopian tube plotted against parity. Chart shows an increase in trend of arterioles with increase in parity.

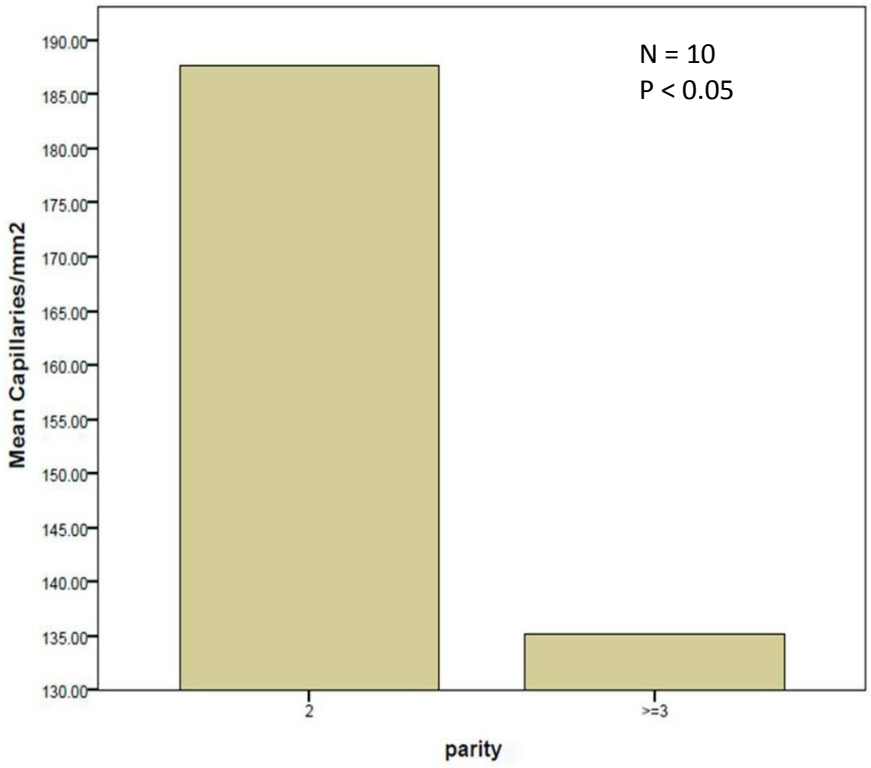


Chart 3: Bar chart of mean number of capillaries per mm^2 of mucosa of the human postpartum Fallopian tube plotted against parity. Chart shows a decrease in trend of capillaries with parity.

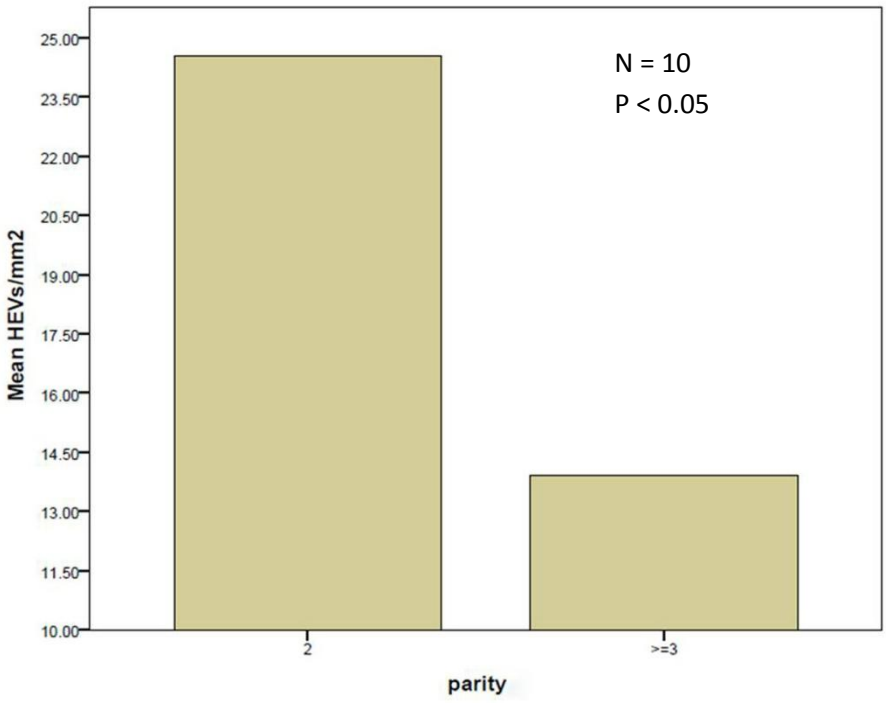


Chart 4: Bar chart of mean number of HEVs per mm^2 of mucosa of the human postpartum Fallopian tube plotted against parity. Chart shows a decrease in trend of HEVs with parity.

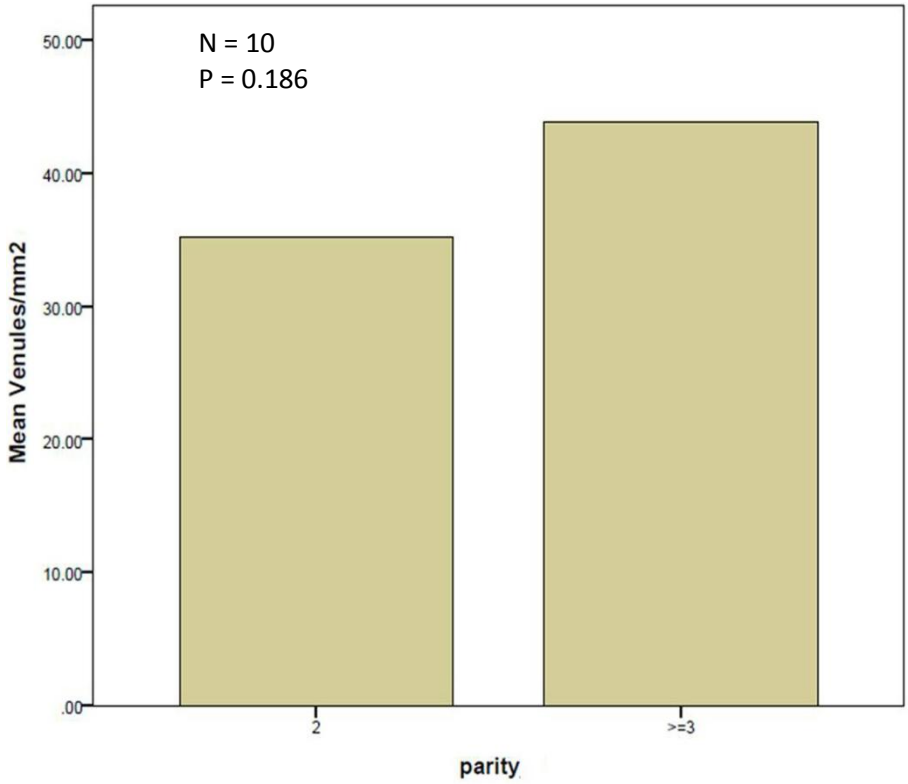


Chart 5: Bar chart of mean number of venules per mm^2 of the mucosa of the human postpartum Fallopian tube plotted against parity. Chart shows an increase in trend of venules with parity.

Intraepithelial lymphocytes:

In the resin embedded 1 µm semi-thin sections of the 10 human postpartum Fallopian tubes stained with toludine blue, the intraepithelial lymphocytes lying within the ciliated columnar epithelium of the mucosa were identified. The cells had either round, oval or irregular shaped, darkly stained, heterochromatic nuclei with a thin rim of cytoplasm around the nucleus. They were arranged either singly or in rows like a 'string of pearls', within the epithelium (Figure 5.9). The IELs were seen at different levels within the epithelium, either close to the basement membrane or

halfway along the epithelium or close to the surface of the lumen (5.11). The mean number of IELs per 100mm length of the basement membrane was 771.30 $\pm$ 300.85 (Table 3). There was a significant positive correlation between the number of HEVs and the IELs with a Spearman's rho correlation coefficient of 0.985, at the level of 0.01 (2-tailed) (Chart 6 and Table 4)

The results of regression analysis show IELs regressing on HEVs. The number of IELs was significantly related to the number of HEVs. For every raise of a single HEV/mm2, there was an increase in the number of IELs/100mm by 42.002 (Table 5)

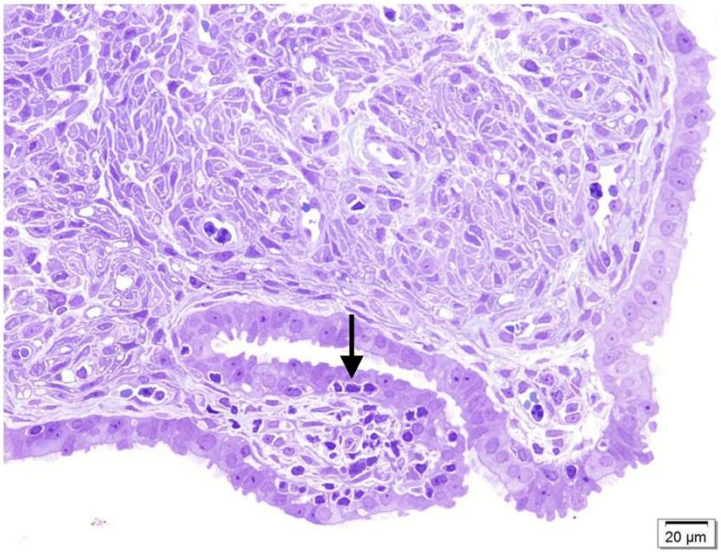


Figure 5.9: A toluidine blue stained section of a mucosal fold of the human postpartum Fallopian tube showing a row of intraepithelial lymphocytes (arrow) along the basement membrane.

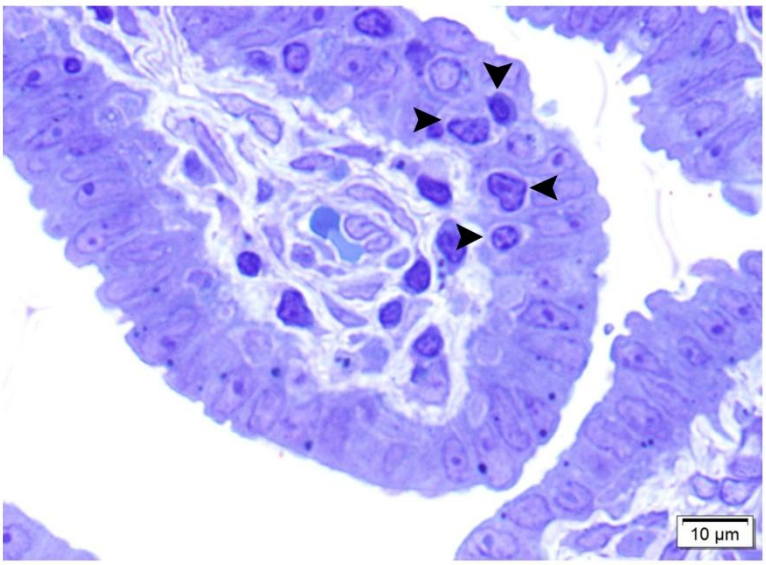


Figure 5.10: A toluidine blue stained section of a mucosal fold of the human postpartum Fallopian tube showing intraepithelial lymphocytes at different levels (arrow heads) of the epithelium.

Table 3: Frequency of number of intraepithelial lymphocytes per 100mm length of the epithelial basement membrane studied under light microscopy.

Mean	Standard deviation	Range	Median	Interquartile range
771.30	300.85	396.00 – 1316.00	735.50	534.50 – 957.75

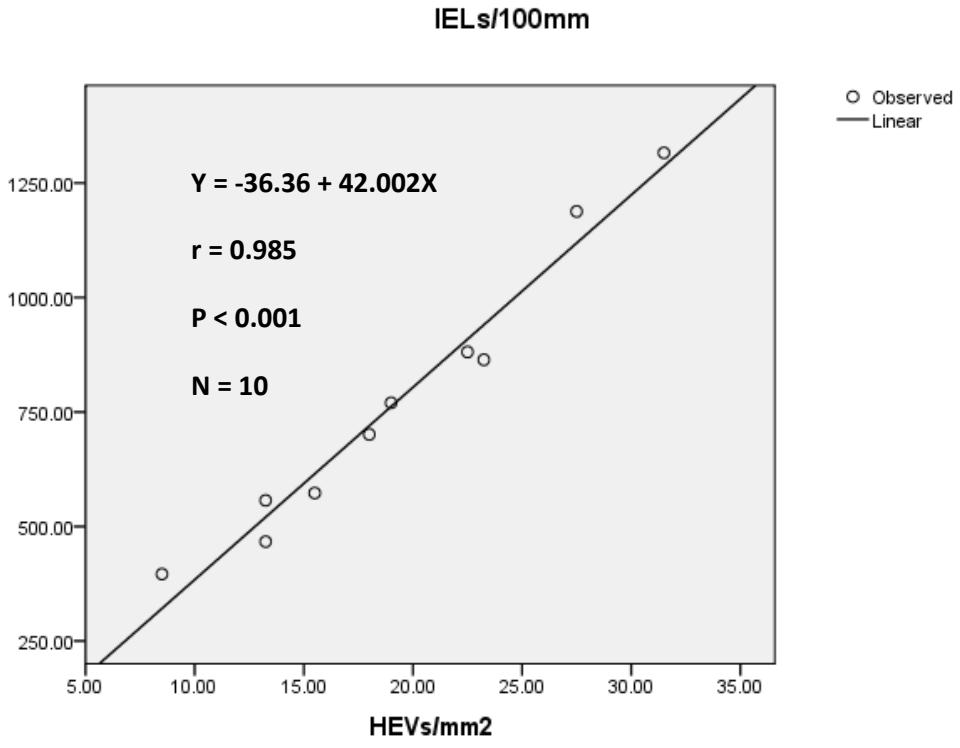


Chart 6: This scatter plot shows a significant linear correlation of the number of intraepithelial lymphocytes (IELs)/100 mm with the number of high endothelial venules (HEVs)/mm^2 in the mucosa of the human postpartum Fallopian tube.

Table 4: Correlation coefficient between HEVs and IELs using Spearman's rho correlation test.

Parameters	IELs	P - value
HEVs	0.985*	< 0.001

Correlation is significant at 0.01 level

Table 5: Analysis of IELs (the dependent variable) against HEVs (the independent variable) of the human postpartum Fallopian tube using simple linear regression.

Regression Coefficient	Standard error (β)	Constant (a)	Standard error (α)	R-square	P-value	95% CI
42.002	2.601	-36.256	52.952	0.970	<0.001	36.007 – 48.004

CI = Confidence interval ; IEL/100 mm = -36.256 + 42.002 HEVs/mm2

ELECTRON MICROSCOPY:

MUCOSA:

Epithelium:

The epithelium of the human postpartum Fallopian tube showed both simple columnar epithelium with cilia (Figure 5.11) and stratified columnar epithelium (Figure 5.12). Epithelium showed ciliated cells, secretory cells and peg cells (Figure5.11).

The ciliated cells were pale with round euchromatic nuclei and usually a single, prominent nucleolus. Their apical surface showed numerous cilia with the typical arrangement of microtubules within them. The supranuclear portion of their cytoplasm contained many globular mitochondria, numerous vacuoles and free ribosomes (Figure 5.13). Two different types of ciliated cells were observed. They were the dark (fig 5.12) and light ciliated cells (Figure 5.13).

The secretory cells were more numerous than ciliated cells. They were also narrower than the ciliated cells, containing microvilli on their apical surface. They had electron dense oval vertically placed nuclei with one or more prominent nucleoli. While a few cells showed highly electron dense vesicles both in their basal as well as apical regions (Figure 5.13), others showed secretory granules which were less electron dense in their supranuclear region (Figure

5.14). In women more than 30 years, apical surface of a few secretory cells also showed microplicae-like structures (Figure 5.15). Peg cells were also dark cells that were seen interpolated between the ciliated and the secretory cells with their apical portion protruding into the lumen (Fig 5.11 and 5.14). A dendritic cell with an indented nucleus and a single cytoplasmic process without intercellular junctions was also seen within the epithelium (Figure 5.16).

Numerous intraepithelial lymphocytes were seen in the epithelium (Figure 5.17 & 5.18). They were irregular shaped cells with numerous cytoplasmic extensions. IELs had either round or oval nuclei with dense, peripherally condensed chromatin and prominent central nucleoli. Their cytoplasm was minimal, forming a rim around the nucleus and showed cytoplasmic extensions making membranous contact with the ciliated and secretory cells of the epithelium. IELs were seen migrating within the epithelium and also seen being expelled into the lumen. In addition to the lymphocytes, eosinophils (Figure 5.19) and plasma cells (Figure 5.20) were also seen within the epithelium. At places the epithelium was found denuded (Figure 5.21). In a specimen belonging to a woman of late reproductive age, the height of the epithelium appeared to be much less than the others (Figure 5.22).

Figure 5.11: A transmission electron microscopic picture showing the mucosa of the human postpartum Fallopian tube lined by simple columnar epithelium with a core of lamina propria. The epithelium shows ciliated cells (C), secretory cells (S) and peg cells (P). The lamina propria shows a capillary (red arrow head) with a red blood cell (*); N - nucleus of ciliated cell.

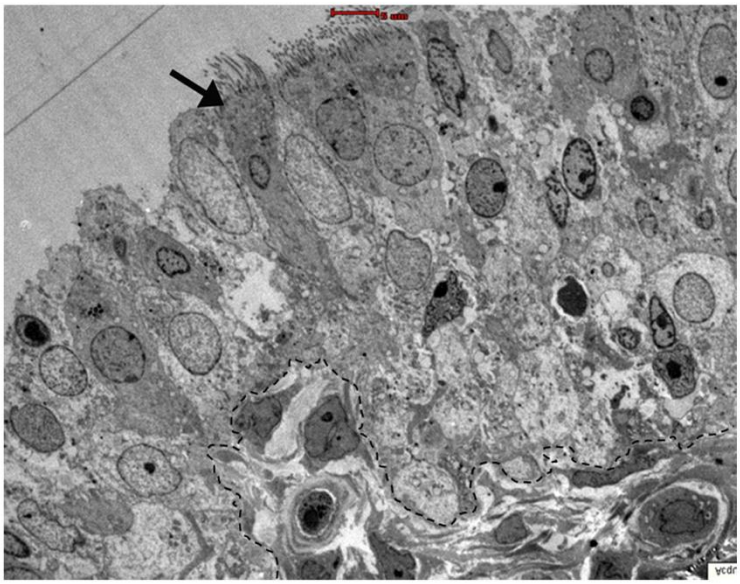


Figure 5.12: A transmission electron microscopic picture showing the stratified columnar epithelium of the mucosa of the human postpartum Fallopian tube. A dark ciliated cell (arrow) is seen as a tall columnar cell with electron dense cytoplasm and a small round nucleus. The dotted line represents the basement membrane.

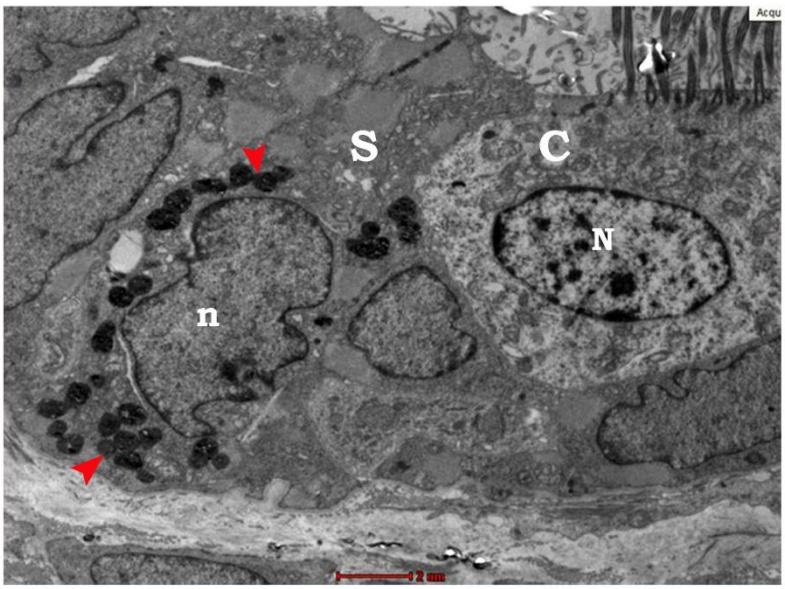


Figure 5.13: A transmission electron microscopic picture of the epithelium of the human postpartum Fallopian tube with secretory (S) and light type ciliated cells (C). The secretory cells contain numerous electron dense secretory granules both in the basolateral and apical regions of the cell (red arrow heads); N and n denote nuclei of the ciliated and secretory cells respectively.

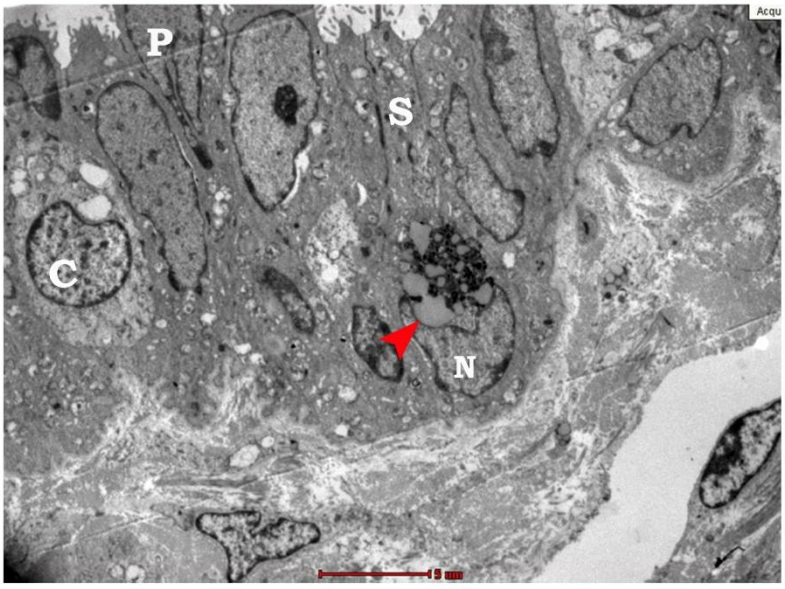


Figure 5.14: A transmission electron microscopic picture of the human postpartum Fallopian tube epithelium showing secretory cells (S), peg cells (P) and ciliated cells (C). The red arrow head indicates less electron dense granules in the supranuclear portion of the cytoplasm of a secretory cell; N - nucleus of the secretory cell.

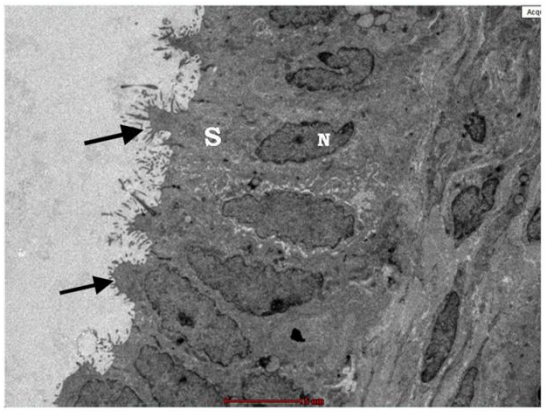


Figure 5.15: A transmission electron microscopic picture of the epithelium of the human postpartum Fallopian tube showing electron dense columnar secretory cells (S) with indented nuclei (N) and prominent nucleoli. The apical surface of these cells shows microplicae-like structure (arrow).

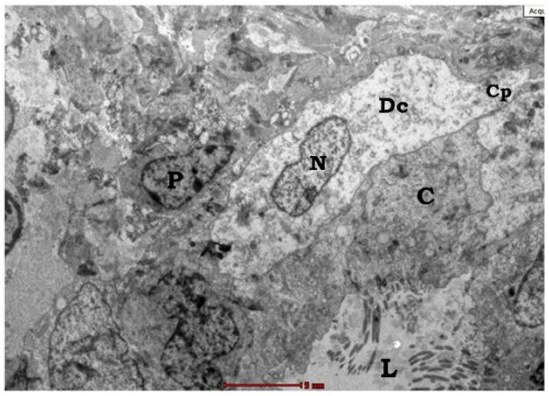


Figure 5.16: The epithelium of the human postpartum Fallopian tube under transmission electron microscopy showing a dendritic cell (Dc) with a single cytoplasmic process (Cp) directed towards the basement membrane. The dendritic cell is pale stained with an oval indented nucleus (N) and a prominent nucleolus; C - ciliated cell, P - peg cell, L - lumen fo the Fallopian tube.

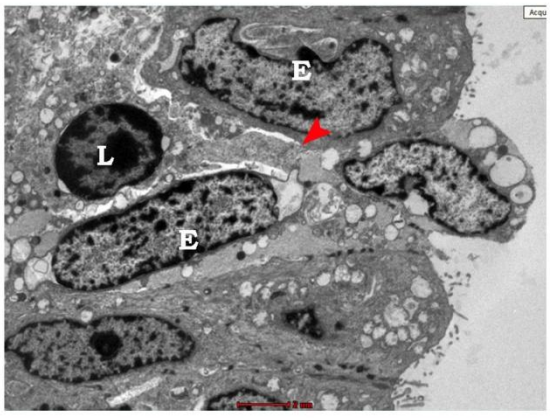


Figure 5.17: The epithelium of the human postpartum Fallopian tube showing the intercellular migration of an intraepithelial lymphocyte (L) between the epithelial cells (E) as seen under transmission electron microscope. The membrane contact between a cytoplasmic extension of the intraepithelial lymphocyte and the epithelial cell membrane is shown by arrow head.

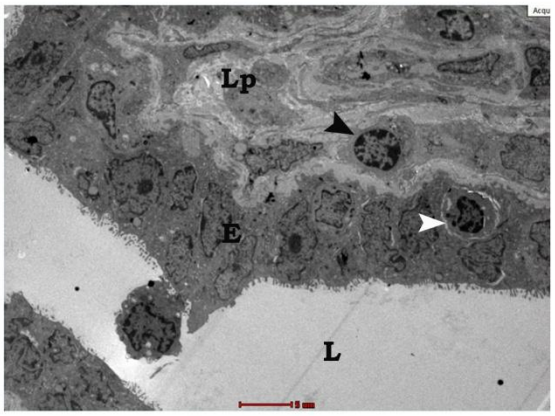


Figure 5.18: The human postpartum Fallopian tube mucosa showing two lymphocytes, one in the lamina propria (black arrow head) and one within the epithelium (white arrow head) seen under transmission electron microscope; L - lumen of the Fallopian tube, E - epithelium, L - lamina propria.

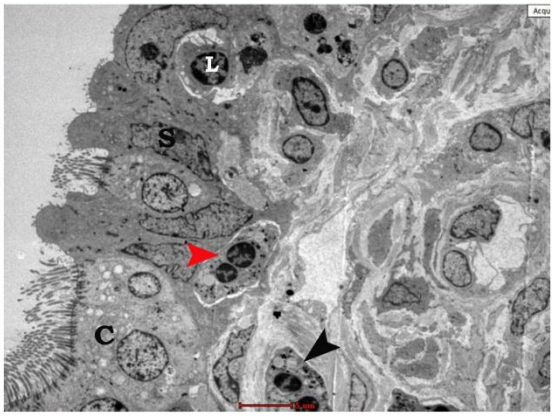


Figure 5.19: A transmission electron microscopic picture of the mucosa of the human postpartum Fallopian tube showing the lining epithelium with ciliated cells (C), secretory cells (S) and intraepithelial lymphocytes (L). Eosinophils are seen one within the epithelium (red arrow head) and the other in lamina propria (black arrow head).

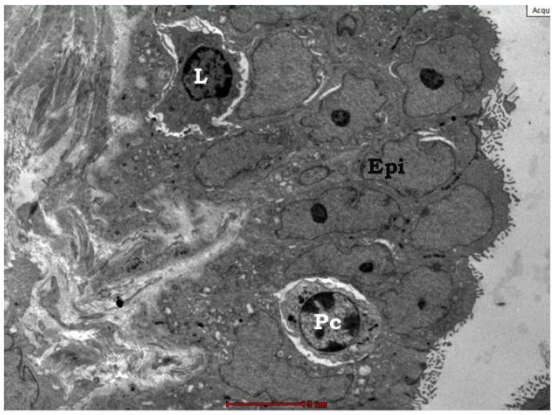


Figure 5.20: The mucosa of the human postpartum Fallopian tube under transmission electron microscopy showing numerous secretory cells with an intraepithelial lymphocyte (L) and a plasma cell (Pc) within the epithelium (Epi).

LAMINA PROPRIA:

The lamina propria of the human postpartum Fallopian tube was edematous and fibrinoid changes were noted. It had numerous lymphocytes (Figure 5.18), plasma cells (Figure 5.23), mast cells (Figure 5.24) and occasionally eosinophils (Figure 5.19). The subepithelium showed numerous dendritic cells, either singly or in rows (Figure 5.25) with their cytoplasmic processes establishing membrane contact with the epithelial cells.

The plasma cells had eccentrically placed nuclei with alternating dark and pale appearing nuclear peripheries and prominent nucleoli. Their cytoplasm was less electron dense than the lymphocytes and showed abundant rough endoplasmic reticulum. Eosinophils in the lamina propria showed bilobed nuclei with cytoplasm containing typical rod shaped crystalline particle containing granules.

Mast cells:

The mucosa contained numerous mast cells in the lamina propria. They were seen widely distributed within the core of the mucosal folds and were usually seen close to blood vessels. The mast cells were round, oval or elongated cells. They usually showed numerous slender (Figure 5.26a) or blunt cytoplasmic extensions (Figure 5.26b). Few cells had no cytoplasmic extensions (Figure

5.26c). These cytoplasmic extensions also made membrane contacts with the adjacent cells of the connective tissue. Their cytoplasm had numerous granules and cellular organelles like mitochondria, golgi complexes, free ribosomes and endoplasmic reticulum. The granules of the mast cells were electron dense, membrane bound and round to oval in shape. The granules contained finer components that were rod-shaped or round with either completely electron dense or completely electron lucent or electron lucent periphery and electron dense centre, lamellar, finely particulated (Figure 5.26c). The mean number of mast cells in the mucosa of the human postpartum Fallopian tube per 1.5625mm^2 area of the mucosa was 40.30 $\pm$ 15.68 (Table 6).

Table 6: Frequency of number of mast cells per 1.5625 mm^2 area of mucosa of the human postpartum Fallopian tube calculated under transmission electron microscope.

Mean	Standard deviation (SD)	Range		Median	Interquartile range
		Minimum	Maximum		
40.30	15.68	20.00	62.00	38.00	(25.25 – 56.00)

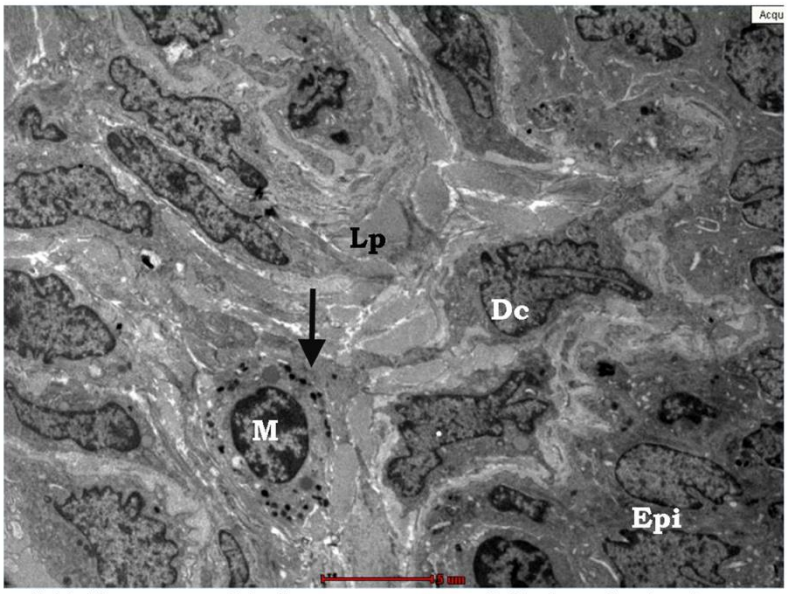


Figure 5.24: The mucosa of the human postpartum Fallopian tube showing a mast cell (M) and a dendritic cell (Dc) in the lamina propria seen under transmission electron microscope. The dendritic cell shows three cytoplasmic processes. One of the dendritic processes is seen establishing membrane contact with the mast cell (arrow).

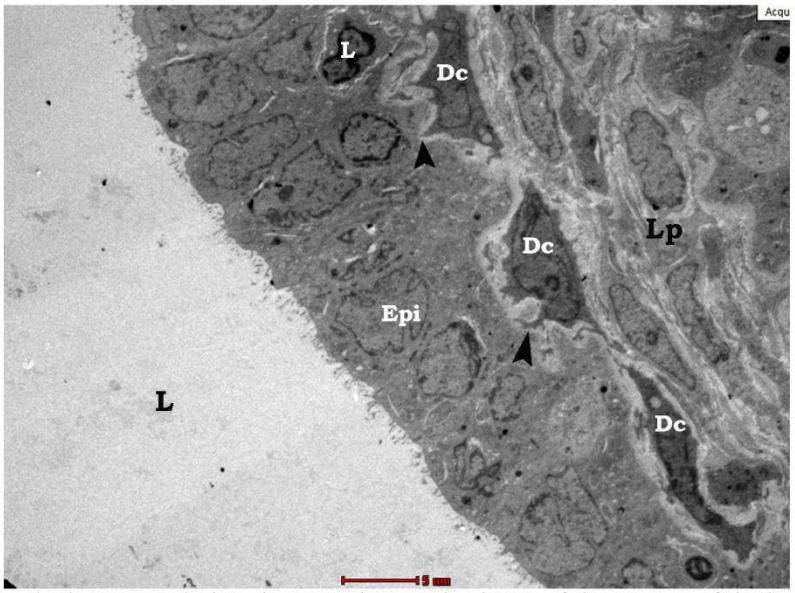


Figure 5.25: A transmission electron microscopic picture of the mucosa of the human postpartum Fallopian tube showing many dendritic cells (Dc) arranged in a row in the subepithelium underlying the basement membrane. These cells show numerous cytoplasmic processes establishing contact with the basement membrane (arrow heads). An intra epithelial lymphocyte (L) is also seen in close association with a dendritic cell; F - fibroblast, L – lumen, Epi – epithelium.

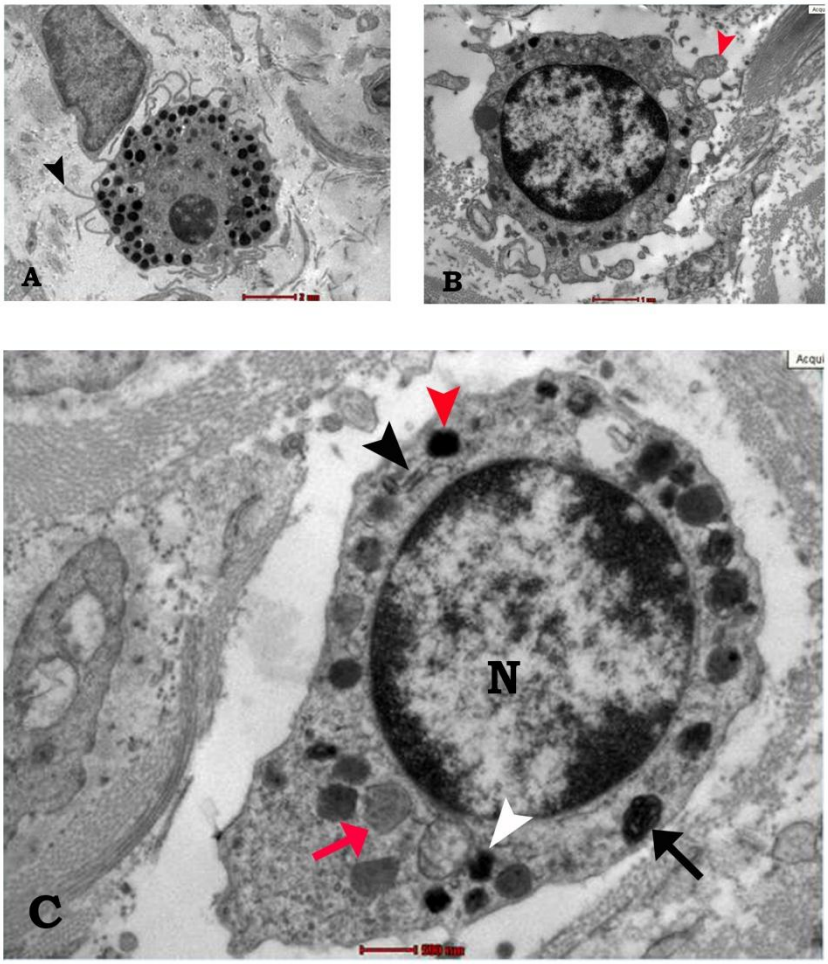


Figure 5.26: A transmission electron microscopic picture of mast cells in the lamina propria of the human postpartum Fallopian tube. A) A mast cell with numerous slender cytoplasmic processes (black arrow head). B) A mast cell with blunt cytoplasmic processes (red arrow head). C) A mast cell with no cytoplasmic processes. The cytoplasm has numerous secretory granules; rod shaped granule - black arrow head, round electron dense granule - red arrow head, round electron lucent granule - red arrow, electron lucent periphery with electron dense centre granule - white arrow head, lamellated granule - black arrow.

Microvessels:

The lamina propria of the human postpartum Fallopian tube showed numerous microvessels including arterioles, capillaries, high endothelial venules and venules, in addition to the collagen fibers, fibroblasts and other immune cells.

Arterioles*:*

The lamina propria showed numerous arterioles. The walls of the arterioles were made up of tunica intima, media and adventitia. The tunica intima showed a single layer of endothelial cells with a clear, highly wavy, less electron dense internal elastic lamina. The endothelial cells protruded into the lumen giving a wavy outline to the lumen. Blood cells were also found within the lumina of the arterioles. The endothelial cells were connected to each other by tight junctions. The tunica media showed a single layer of smooth muscle cells. The smooth muscle cells had electron dense cytoplasm with electron dense nuclei with thin, condensed chromatin in the periphery. The tunica adventitia was thin with fibroblasts and collagen bundles. The fibroblast cells had cytoplasm which was less electron dense and oval to elongated euchromatic nuclei (figure 5.27).

Capillaries:

Numerous capillaries were seen in the subepithelial region of the mucosa. Capillaries were lined by flattened endothelial cells enclosing pericytes within their basal lamina. Endothelial cells showed tight junctions between them. Fenestrated capillaries were not observed. Their cytoplasm contained numerous pinocytotic vesicles and their surfaces showed numerous elevations and caveolae. Red blood cells were seen within the capillaries but they did not have the typical biconcave appearance. The basal lamina was, in turn, surrounded by fibroblast cells and dense collagen bundles (Figure 5.28).

High endothelial venules (HEVs):

Under electron microscopy, the HEVs were easily identified by their large cuboidal endothelial cells having faintly electron dense cytoplasm. The endothelial cells were connected to each other by tight junctions. Their cytoplasm contained rounded or lobular mitochondria and prominent Golgi bodies. They had numerous free and clustered ribosomes and sparse endoplasmic reticulum. The luminal surfaces of the endothelial cells were usually smooth. Their cytoplasm also contained pinocytotic vesicles. The electron dense, typical Wiebel-Palade bodies that are characteristic of the endothelium were not seen. The nuclei of the endothelial cells were large, lobular or irregular with loose chromatin condensed at the

periphery, each usually with a single, electron dense prominent nucleolus (Figure 5.29a). Pericytes were also seen in association with the HEVs, enclosed within the basal lamina of the endothelial cells (Figure 5.29b).

The lumen of the HEVs showed the presence of platelets (Figure 5.29a), neutrophils (Figure 5.29b), red blood cells and lymphocytes (Figure 5.29c). The lymphocytes seen within the lumen of the HEVs had membrane contact with the endothelial cell membranes (Figure 5.29c). Transendothelial migration of lymphocytes through the high endothelial venules was also observed (Figure5.29d).

Venules:

The mucosa showed numerous dilated, thin walled venules showing tunica intima, media and adventitia. The tunica intima of each was lined by a single layer of flattened endothelial cells with a smooth surface giving the lumen a smooth outline. The typical internal elastic lamina seen in arterioles was absent. The tunica media contained a single layer of smooth muscle cells and the tunica adventitia was thin, containing fibroblasts and less dense collagen bundles. Numerous blood cells namely red blood cells, lymphocytes, platelets, basophils and neutrophils were seen within their lumina (Figure 5.30a). Activated platelets were also seen within the lumen of a venule (Figure 5.30b).

Figure 5.27: A transmission electron microscopic picture of an arteriole (A) in the lamina propria (Lp) of the human postpartum Fallopian tube with a single layer of endothelium (E) surrounded by a single layer of smooth muscle cells (Sm), in turn surrounded by connective tissue with fibroblasts (F) and collagen bundles (Co). A capillary (*) and a mast cell (M) are also seen adjacent to the arteriole.

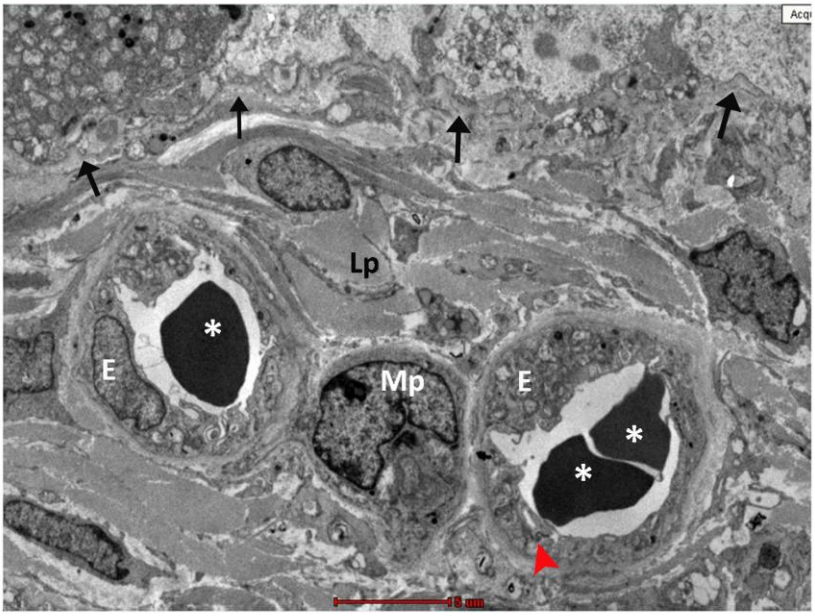


Figure 5.28: A transmission electron microscopic picture of two capillaries with an intervening macrophage (Mp) is seen lying in the lamina propria (Lp) of the human postpartum Fallopian tube subepithelially. Note the flattened capillary endothelial cells (E) with cytoplasm showing numerous pinocytotic vesicles, surface processes and caveolae. The red arrow head indicates a tight junction between the endothelial cells; (*) - red blood cell in the lumina of the capillaries, arrows - basement membrane of the epithelium.

Figure 5.29a: A transmission electron microscopic picture of a high endothelial venule (HEV) lined by cuboidal endothelium (E) in the lamina propria of the human postpartum Fallopian tube showing a platelet (P) within the lumen. Epithelium (Epi) with an intraepithelial lymphocyte (arrow) is seen. A dendritic cell (Dc) is seen in association with the HEV.

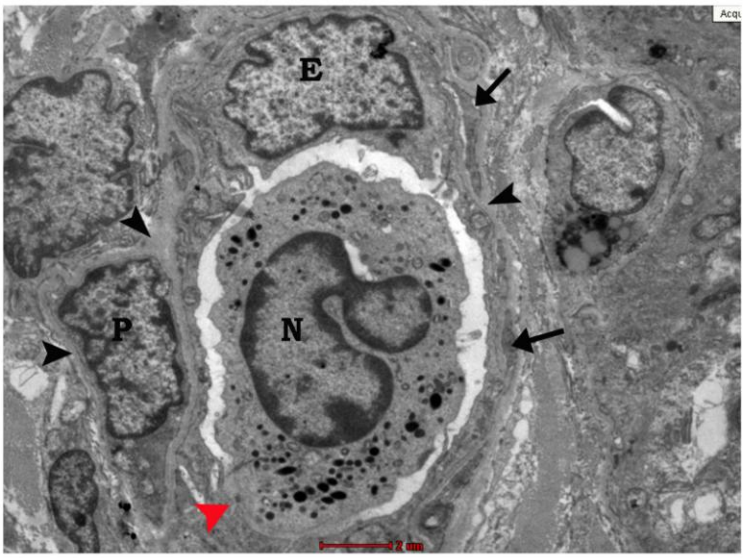


Figure 5.29b: A high endothelial venule (HEV) in the lamina propria of the human postpartum Fallopian tube seen under transmission electron microscope. The lumen of the HEV shows a neutrophil (N) establishing membrane contact (red arrow head) with an endothelial cell (E). A pericyte (P) is enclosed within the basal lamina (black arrow heads) of the HEV. Arrows represent reticular sheets of the basal lamina.

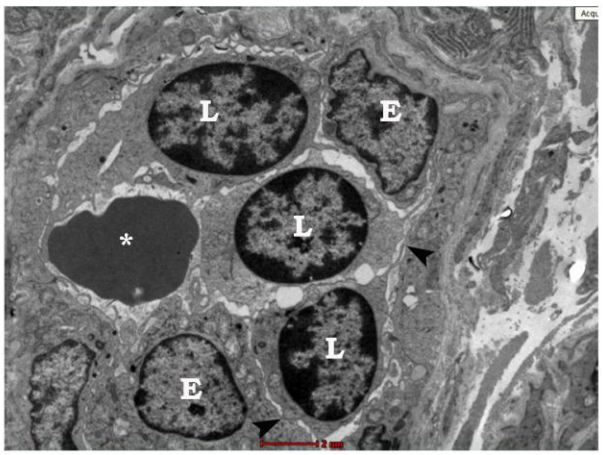


Figure 5.29c: A transmission electron microscopic picture of a high endothelial venule in the lamina propria of the human postpartum Fallopian tube with a red blood cell (*) and lymphocytes (L) in its lumen. The cytoplasmic processes of the lymphocytes establish multiple contacts (arrow heads) with the luminal surface of endothelium (E).

Figure 5.29d: A transmission electron microscopic picture of a high endothelial venule in the lamina propria of the human postpartum Fallopian tube showing a cuboidal endothelial cell (E) with globular mitochondria (red arrow heads) and numerous vesicles (*). A lymphocyte (L) enclosed within the cytoplasm of the endothelial cell suggesting transendothelial migration is also noted.

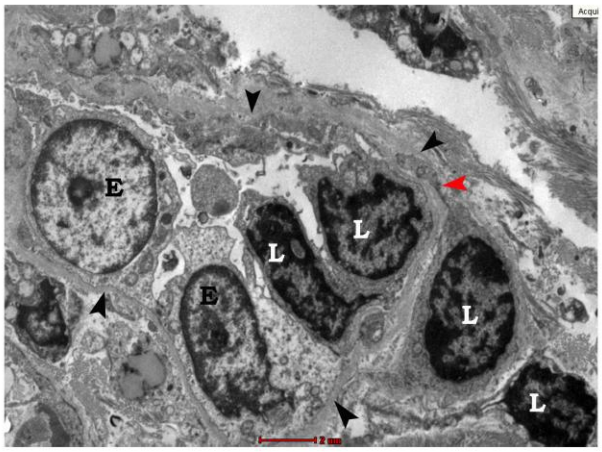


Figure 5.29e: A high endothelial venule (HEV) with cuboidal endothelium (E) in the lamina propria of the human postpartum Fallopian tube showing multiple lymphocytes (L) migrating through its wall. Lymphocytes are seen in the process of migration through the wall of the HEV. The red arrow head represents a cytoplasmic process of the lymphocyte emerging out from the wall of the HEV. Black arrow heads represent the basal lamina of the endothelium.

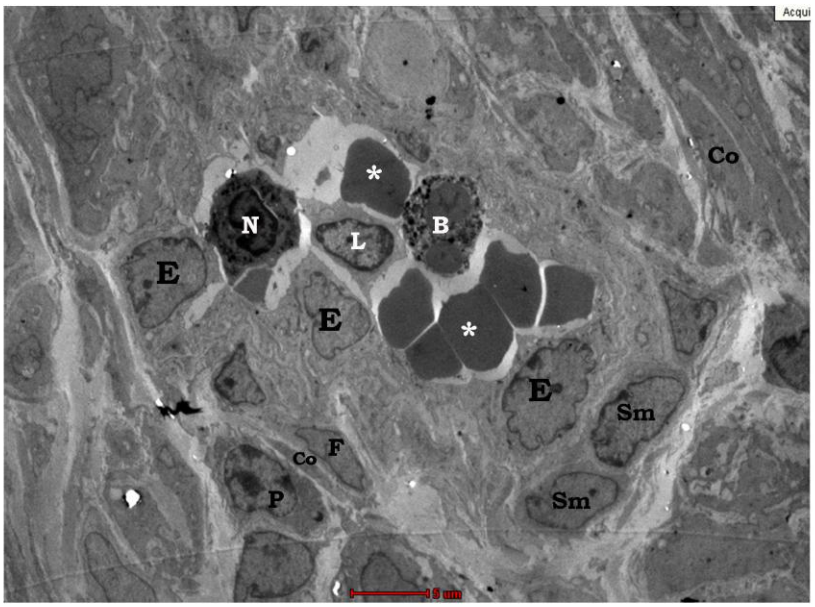


Figure 5.30a: A transmission electron microscopic picture of a venule in the lamina propria of the human postpartum Fallopian tube. The venule shows tunica intima with endothelial cells (E), tunica media with smooth muscle cells (Sm) and tunica adventitia with fibroblasts (F) and collagen bundles (Co). Lumen contains a neutrophil (N), a basophil (B), a lymphocyte (L) and red blood cells (*). A plasma cell (P) is also seen in association with the venule in the lamina propria.

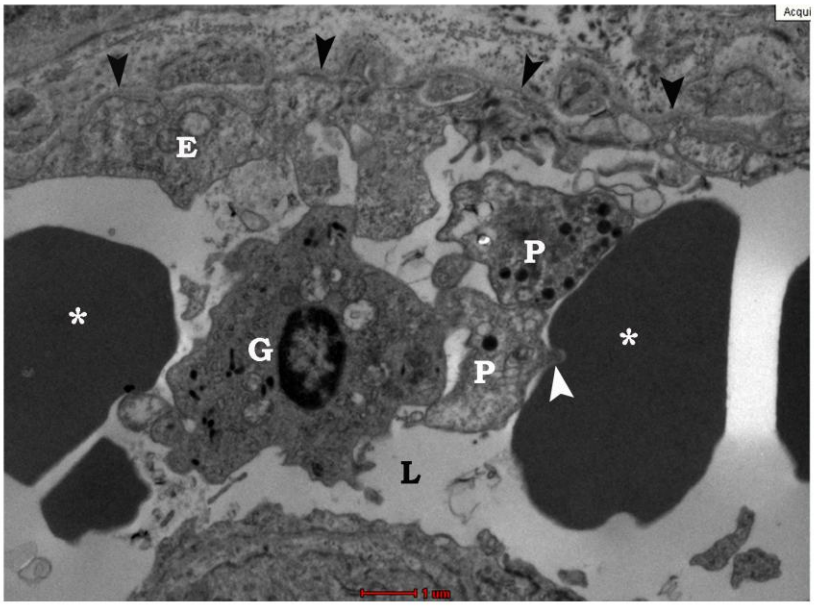


Figure 5.30b: A transmission electron microscopic picture showing a part of a venule in the lamina propria of the human postpartum Fallopian tube with red blood cells (*), a granulocyte (G) and activated platelets (P) that shows numerous cytoplasmic projections (white arrow head). Black arrow heads represent the basal lamina of the endothelium.

ANTIGEN PRESENTING CELLS ASSOCIATED WITH BLOOD VESSELS:

Dendritic cells were predominantly located in the subepithelial region. These cells were seen arranged singly or in rows just beneath the basement membrane of the epithelium making numerous membrane contacts with it (Figure 5.25). The dendritic cells showed single to multiple cytoplasmic processes and oval highly indented nuclei. They were seen in association with the HEVs (Figure 5.31) in the lamina propria. They were also seen in apposition with mast cells in the lamina propria (Figure 5.24). Plasma cells were also seen in association with capillaries, HEVs (Figure 5.32) and venules (Figure 5.30a).

TELOCYTES:

The interstitial Cajal like cells (ICLCs), called telocytes, were seen located in the mucosal and the muscular layers of the human postpartum Fallopian tube. These cells were found abundantly in the lamina propria, predominantly underlying the basement membrane of the epithelium. Their thin podomeres and dialated podoms with caveolae and mitochondria were clearly seen (Figure 5.33a). These cells were also seen in association with the blood vessels (Figure 33b). The telocytes seen in the muscular layer showed triangular cell bodies with heterochromatic nuclei,

prominent nucleoli. A thin rim of cytoplasm surrounded each nucleus. Three thin and long cytoplasmic processes were seen extending from the cell body (Figure 33c).

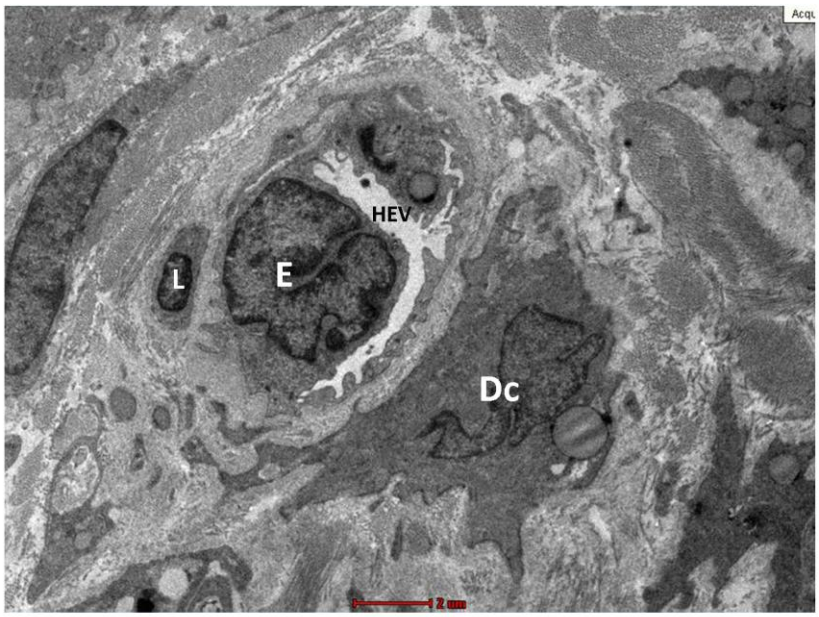


Figure 5.31: A high endothelial venule (HEV) in the lamina propria of the human postpartum Fallopian tube seen under transmission electron microscope. A lymphocyte (L) is seen after migration through the wall of the HEV. A dendritic cell (Dc) is seen in association with the HEV.

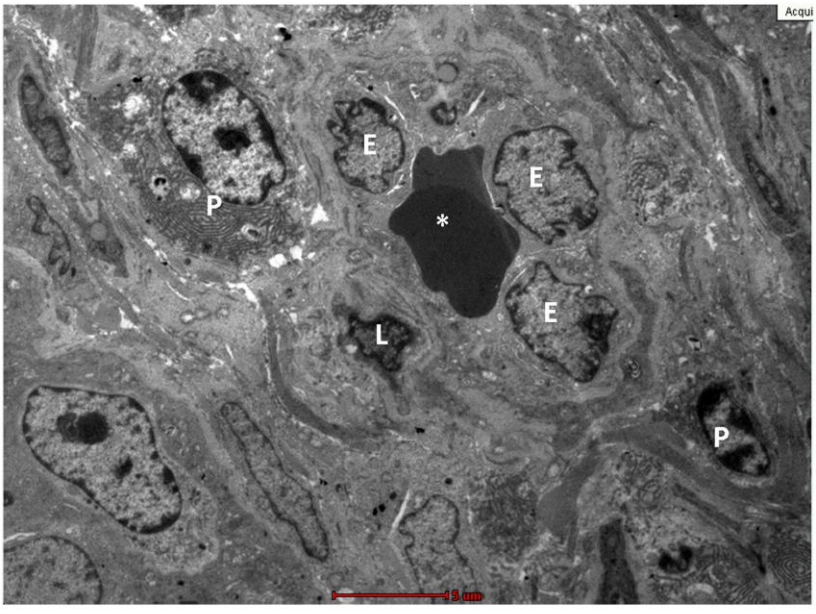


Figure 5.32: A transmission electron microscopic picture of a high endothelial venule in the lamina propria of the human postpartum Fallopian tube seen in association with plasma cells (P) and a lymphocyte (L); (*)- red blood cell, E - endothelium.

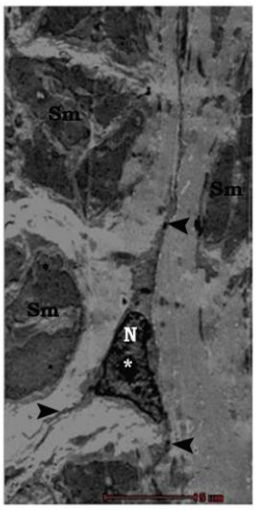


Figure 5.33a: A transmission electron microscopic picture of a telocyte in the muscular layer of the human postpartum Fallopian tube showing a triangular cell body with a heterochromatic nucleus (N) and a prominent nucleolus (*) with a thin rim of cytoplasm around the nucleus. Three long slender cytoplasmic processes are seen (arrow heads); Sm – smooth muscle bundle.

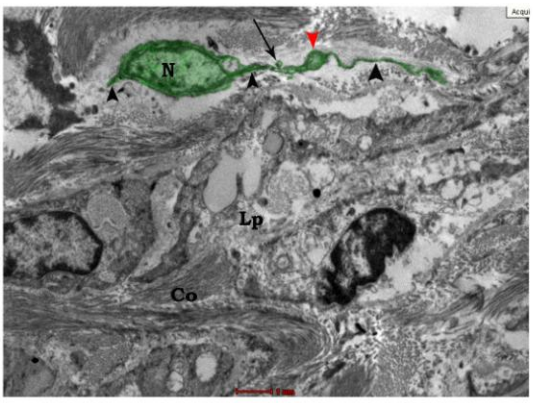


Figure 5.33b: A TEM picture of the lamina propria (Lp) of the human postpartum Fallopian tube. A telocyte has been highlighted in green. It shows an oval cell body and nucleus (N) with a thin rim of cytoplasm. Thin cytoplasmic process or podomere (black arrow heads) are seen extending from the cell body with dilated regions or podoms (red arrow head); Co – collagen bundle, black slender arrow - exocytotic vesicle.

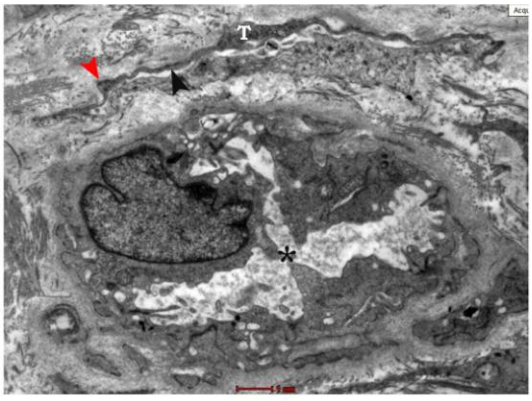


Figure 5.33c: A transmission electron microscopic picture of a HEV (*) in the lamina propria of the human postpartum Fallopian tube seen in association with a process of a telocyte (T). The process shows a thin podomere (black arrow head) with a dilated podom (red arrow head).

6. DISCUSSION

MUCOSA OF THE FALLOPIAN TUBE:

EPITHELIUM:

The human Fallopian tube has innermost mucosa, middle muscular layer and outer serosal layers. The mucosa is thrown into folds and these folds are more numerous and highly branched in the ampulla, converting the lumen into a shape that resembles a labyrinth (2–4). Each fold has a lining epithelium that is simple columnar with ciliated cells , secretory cells, peg cells and indifferent cells (101), and a core of lamina propria which is made of loose connective tissue richly supplied by blood vessels and lymphatics (2–4). Ciliated cells are predominantly seen in the ampulla and fimbriae. The secretory cells are taller than the ciliated cells and are more numerous in the mid cycle. Their secretions have been found to nourish the gametes and the developing blastocyst (46).

In the present study, a higher number of secretory cells was noted in the epithelium of human postpartum Fallopian tube as compared with that of ciliated cells. The ratio of the numbers of ciliated and secretory cells is known to depending on the hormonal status. Estrogen induces ciliogenesis and increase in cell size and secretion, whereas progesterone causes decrease in cell size and

deciliation (50,52). In the present study also, secretory cells were more in number than the ciliated cells. The secretory cells had both electron dense and less electron dense secretory granules in the cytoplasm. Though most of the epithelium was lined by columnar epithelium either simple or stratified, the Fallopian tube from a 39 year old woman with parity four showed very low cuboidal epithelium. In another 35 year old woman, epithelium was denuded and deciliation was markedly noted. This could be due to long term exposure of the Fallopian tube epithelium to progesterone, or age could be a factor involved.

True microplicae are the surface modifications that occur in the stratified squamous epithelia of cornea, oesophagus, pharynx, ectocervix, vagina and vulva where they are exposed to the external environment and are exposed to repeated abrasions (53). Correr et al. 2006 reported that secretory cells show gradual shortening of microvilli and form microplicae-like structures 20 – 30 years after menopause (53). However, in the present study, microplicae-like structures were seen in postpartum Fallopian tube of two women above 35 years.

INTRAEPITHELIAL LYMPHOCYTES:

As any other mucosal associated lymphoid tissues like gut, nasal mucosa, bronchus, urinary system, conjunctiva, lacrimal gland (16). The female genital tract also has intra-epithelial lymphocytes

(mainly T- lymphocytes) and lymphoid aggregations. They have been reported in uterus (102), vagina & cervix (103). They were initially mistaken for basal cells and were also seen populating the mucosa of the Fallopian tubes (64–66,103–106). The IELs were found to be exclusively situated in the basal regions of the epithelium (65,104). In the present study, many IELs were seen within the epithelium of the postpartum Fallopian tubes. They were seen predominantly in the basal regions but were also seen at different levels of the epithelium and extruding into the lumen of the Fallopian tube, in contrary to earlier reports (65).

IELs are considered to be migratory lymphocytes that were found to transiently occupy strategic positions against surface antigens (102). They are predominantly cytotoxic T cells and suppressor T cells [(CD8+) and (CD3+) cells] (61,65,104). Rarely macrophages are also seen within the epithelium (104,106) but T helper cells (CD4+), plasma cells and B cells have not been seen (34,66,104). In addition to the Fallopian tube, it has been reported in other female reproductive organs like cervix and vagina that the IELs are cytotoxic T cells (103). However, the primary MALT type B cell lymphomas of the Fallopian tube contained B lymphocyte lineage (CD 20) infiltrating the epithelium and it has been suggested that these tumours should be kept in mind if lymphoid cells of B cell lineage are observed in surgical specimens of Fallopian tubes (107).

In the present study, in addition to the IELs, plasma cells were seen in the postpartum Fallopian tube epithelium under electron microscopy. Thus, the types of IELs in postpartum Fallopian tube need to be studied further using specific markers for the various lymphocytes.

The numbers of intraepithelial lymphocytes in different parts of the Fallopian tube and during different phases of the menstrual cycle have been disputed. In the uterus of rats, IELs were more in the diestrus phase, less in the proestrous phase and lowest in early pregnancy (102). In one report of the human Fallopian tube, there was no significant difference in the number of IELs in the ampullary part in different phases of menstrual cycle and in tubectomy specimens (65). However in few others, IELs were seen more numerous in the secretory phase compared to the proliferative phase (66,104) and were more in the reproductive age group when compared with postmenopausal women (104). During pregnancy, in the human Fallopian tube, the IELs were much higher than during the phases of menstrual cycle and were seen arranged as a “string of pearls” (104). The number of IELs were less in tubal pregnancy compared to nonpregnant women of the reproductive age group (104). In the present study the mean number of IELs per 100mm was 771.30 $\pm$ 300.85. They were seen singly or in rows.

The presence of IELs in the mucosa of the Fallopian tube was considered to be physiological (65,108) and not due to salphigitis (108). It was proposed that they might act as a first line of defense against infections and thereby prevent infertility (61,65). They were also found to play an important role in immune tolerance, facilitating the transport of sperm and blastocyst without triggering a local immune response (64,65).

All the IELs were found to be positive for estrogen receptor β in addition to the stromal cells and the vascular endothelial cells. Estrogen receptor β positive IELs and stromal cells were found to be abundant in tubal pregnancies (66).

Interaction of IELs with the neighbouring epithelial cells through multiple cytoplasmic extensions was noted in our study. The close apposition of plasma membranes of the intraepithelial lymphocytes and the epithelial cells is due to the complex inter-digitations and laminations. These arrangements significantly increase the total contact area between the two cells. Though multiple contacts were seen between the IELs and neighbouring epithelial cells, no cell junctions were observed at the site of interaction in the present study. These findings were consistent with earlier reports (63,105).

High endothelial venules are the migratory route of naive lymphocytes in the secondary lymphoid organs and tissues (68).

In the present study, there was a significant positive correlation between the number of HEVs and the IELs with the number of IELs significantly regressing on the number of HEVs. The presence of IELs, HEVs and the manner of interaction of IELs with the neighbouring epithelial cells proves that the Fallopian tube is indeed a member of the MALT.

LAMINA PROPRIA:

In the present study, the lamina propria of the human postpartum Fallopian tube showed stromal edema and fibrinoid degeneration. Numerous blood vessels were seen with lymphocytic, granulocytic and mast cell infiltration in accordance with previous studies (108,109). No lymphoid aggregations were found in the ampullae of the postpartum Fallopian tubes studied.

MICROVASCULATURE:

Study of the microvasculature of any viscera is important as it can be an indicator of pathological changes. Microvascular diseases are now a major burden in the world. Some of the conditions that predispose to microvascular diseases are high blood pressure, diabetes, autoimmune disorders and certain genetic disorders. Several studies have been done on the microvascular diseases of the heart, eye (retina), brain, kidney, skin and lung (110,111).

Pathological changes in the structure of microvessels were found responsible for the degeneration of cardiac muscle leading to cardiomyopathy (112). Determination of the number of microvessels in tissues is of great importance in the assessment of its metabolic function. Microvascular density is used as one of the predictors to assess tumour growth and prognosis (113). Metabolically active tissue contains abundant capillaries (114). The vascularity of any tissue can be accurately determined by the estimation of the capillary density based on an actual count of the capillaries in a given volume of the tissue (23).

The microvasculature of the Fallopian tube includes the arterioles, capillaries, postcapillary venules (also called HEVs) and venules. The microvascular architecture of Fallopian tube plays a vital role in the transport of ovum lie within the tube and also the zygote to the uterine cavity. It has been proposed that a derangement in this architecture may lead to the disruption of their function which may predispose to ectopic pregnancy (115).

A previous study of the microvasculature of the Fallopian tube in virgin rabbits using corrosion vascular casts with scanning electron microscopy has shown that the ampullary part has few arterioles in the mucosa that terminate in capillaries in the apex of the folds and the density of the sub-epithelial capillary plexus was less. The veins were seen to originate high in the plical core. Based

on the diameters of the vessels, it was hypothesized that the arteriolar supply is more in the isthmus for net fluid secretion, and the venous drainage more in ampulla for net fluid absorption, that helps in the acceptance of the ovum at the fimbrial end during ovulation (58).

In the present study, the quantification of the microvessels of the mucosa of ampullary part of the human postpartum Fallopian tube under light microscopy revealed that the capillaries were the most abundant vessels seen followed by venules, arterioles and HEVs, in that order. Statistically significant mean differences were observed between the capillaries were compared with arterioles, venules and HEVs. The capillaries and HEVs were seen predominantly in the subepithelium of the apical and intermediate portions of the mucosal folds. Arterioles and venules were seen mostly in the basal and intermediate portions of the mucosal folds. Significant positive correlation was found between arterioles and parity, whereas the capillaries and HEVs were negatively correlated with parity.

The capillaries studied under electron microscopy showed that they were all of continuous type. Fenestrations were absent. Fenestrations are under hormonal influence. Progesterone decreases the fenestrated vessels in the ampulla of the postpartum Fallopian tube (33).

All secondary lymphoid tissues have a specialized vascular channel called high endothelial venules (HEVs) which are lined by cuboidal endothelium. They act as a migratory route for the circulating immune cells to reach lymphoid tissues (28,70–73). Though the Fallopian tube is considered as a member of MALT, Otsuki et al. reported that there are no HEVs in the Fallopian tube and lymphatic capillaries were proposed as the migratory route of the lymphocytes (34). However, the present study showed many high endothelial venules (mean 19.23 $\pm$ 7.05) in the mucosa of the postpartum Fallopian tube both in light and electron microscopy and there was a significant positive correlation between the number of IELs and HEVs.

Under electron microscopy, the HEVs were lined by cuboidal endothelium surrounded by layers of concentrically arranged reticular cell plates. Pericytes were also seen in association with the HEVs. Blood cells including red blood cells, lymphocytes and neutrophils were seen in their lumina. Transendothelial migration of lymphocytes was seen. Naive T and B cells are known to migrate through HEVs by the steps described as rolling, sticking, crawling and trans migration (29). In the present study, the adherence of the cytoplasmic processes of the lymphocytes to the luminal surface of the endothelium was noted. HEVs are known to specifically express adhesion molecules called vascular addressins,

which mediate selective lymphocyte attachment to the HEVs (69). The interaction of lymphocytes to the endothelium of HEVs was reported to be mediated by L- selectin which mediates the tethering and crawling processes of lymphocytes (29). After the transendothelial migration, the lymphocytes leave the HEVs by passing through the successive laminations of the reticular plates (32).

In addition to lymphocytes, neutrophils were seen in association with HEVs in the present study, contrary to earlier reports that neutrophils are not associated with the walls of the HEVs (116). It was presumed that under normal conditions, the endothelium of the HEVs prevent the migration of leukocytes other than lymphocytes. HEV-neutrophil association was commonly seen in lymphnodes from newborn rats in the state of prevailing immune incompetence (117). The association of neutrophils with HEVs needs to be further explored.

MAST CELLS:

Recent studies have shown that mast cells play a crucial role in innate immunity (79). In the present study, numerous mast cells were observed in the mucosal and muscular layers of the postpartum Fallopian tube under both light and electron microscopy. Most of the mast cells in the lamina propria were seen

closely related to the blood vessels. The study of mast cells in salivary gland tumours has revealed that mast cell density was significantly increased in mucoepidermoid carcinoma and its aggressive behaviour was attributed to this. A possible role of mast cells in angiogenesis has also been postulated (118). In the uterus, there was no apparent difference in the number of mast cells found between normal, proliferative and secretory phase endometria. They were seen exclusively in the basal layer of the endometrium and it was suggested that the relative paucity of mast cells in the functional layer might contribute to the immune tolerance of the gestational endometrium during implantation of the blastocyst (87). The increased number of mast cells in IUCD users might be a factor in the pathogenesis of pelvic inflammatory disease and the ectopic pregnancies that occur in IUCD users (88).

Tryptase positive mast cells have been observed both in the mucosal and muscular layers of the Fallopian tube (109). Tryptase, an enzyme secreted by mast cells, has an inhibitory effect on sperm motility (83) and an increase in mast cell numbers has been reported to cause male infertility (119). In the present study, large number of mast cells was demonstrated in the lamina propria and muscular layers of the human postpartum Fallopian tube. Thus the presence of large number of mast cells in the postpartum Fallopian tube could be a factor for postnatal infertility and needs to be studied further.

ANTIGEN PRESENTING CELLS AND THEIR ASSOCIATION WITH BLOOD VESSELS:

Antigen presenting cells are those specialized cells that capture microbes and antigens and present them to the T- lymphoyctes after processsing them to set an immune response. They include dendritic cells, macrophages (89) and B lymphocytes (91). Dendritic cells are the professional antigen presenting cells which are usually seen interdigitating within the epithelium and lamina propria, for antigen capture. They are seen both in lymphoid and non lymphoid tissues (89).

In the present study, many dendritic cells were seen in the lamina propria, predominantly in the subepithelium, arranged singly or in rows. This is consistent with a previous study done in our department using immunostaining for their markers. Migration of lymphocytes through HEVs was noted. The lymphocytes after entering the perivascular space, encounter the dendritic cells that are accumulated around the HEVs to set an immune response. Other immune cells like macrophages and plasma cells were also seen in association with the microvessels.

TELOCYTES:

Telocytes in the human postpartum Fallopian tube were demonstrated in the present study, mainly in lamina propria and in between smooth muscule fibres. In the muscular layer, they are believed to act as stretch receptors (sensors) or as pacemakers controlling Fallopian tube peristalsis (98). While telocytes have been seen associated with blood vessels and immune cells in the uterus, there was no such association observed in Fallopian tube (98). In contrary, in the present study, telocytes were seen in association with the blood vessels of the lamina propria of postpartum Fallopian tube thereby suggesting their role in the complex interplay between the telocytes and immune cell trafficking through the vessels.

To summarize, in this study, the number of microvessels in the mucosa of the postpartum Fallopian tube were quantified. High endothelial venules were demonstrated. Although HEVs are said to develop in tissues with chronic inflammation or cancer, our demonstration of HEVs in the postpartum Fallopian tube shows the needs for a better understanding of its role in immune trafficking. It is hoped that the data from this study may provide some insight into the understanding of microvessels in the Fallopian tube under the influence of hormones during pregnancy and their association with immune cells.

7. CONCLUSIONS

1. The epithelium of postpartum Fallopian tube is lined by both simple columnar and stratified columnar epithelium.
2. The epithelium of human postpartum Fallopian tube showed predominantly secretory cells when compared to peg cells and ciliated cells.
3. Secretory cells of biopsies from women in the late reproductive age showed microplicae-like structures on their apical surfaces.
4. A clear cell variant of ciliated cell was noted under light microscopy.
5. The mean number of IEL per 100mm length of the basement membrane of the epithelium was 771.30 $\pm$ 300.85.
6. Significant positive correlation between the number of HEVs and the IELs with a Spearman's rho correlation coefficient of 0.985, at the level of 0.01 was found.
7. By regression analysis it was found that for every single increase in the number of HEV/1mm^2, the number intraepithelial lymphocytes/100mm of epithelium increases by 42.002.
8. The mean number of arterioles per square millimetre area was 29.23 $\pm$ 6.87.
9. The mean number of capillaries per square millimetre area of the mucosa was 161.38 $\pm$ 36.86.

10. The mean number of the mucosal high endothelial venules per square millimetre area was 19.23 $\pm$ 7.05.
11. The mean number of venules per square millimetre area was 39.50 $\pm$ 9.96.
12. Statistically significant differences were found between the mean number of capillaries when compared with arterioles, venules and HEVs ($P < 0.001$).
13. Arterioles showed a statistically significant increase ($p < 0.05$), whereas the capillaries ($p < 0.05$) and HEVs ($p < 0.05$) showed a statistically significant decrease with parity. Venules showed increasing trend with parity but was not statistically significant.
14. No significant correlation was found between age and microvessels.
15. The mean number of mast cells in the mucosa of the human postpartum Fallopian tube per 1.5625mm^2 area of the mucosa was 40.30 $\pm$ 15.68 studied under TEM.
16. No obvious structural changes were noted in the microvessels under TEM.
17. There were no fenestrated capillaries in the human postpartum Fallopian tube mucosa.
18. High endothelial venules were seen and the transendothelial migration of the lymphocytes through it was also observed under TEM.

19. Neutrophils were seen to be associated with high endothelial venules.
20. Antigen presenting cells like dendritic cells, macrophages and B cells were also associated with the microvessels.
21. Immune cells like dendritic cell, lymphocytes, eosinophils and plasma cells were also observed in the epithelium of the human postpartum Fallopian tube.
22. Telocytes were seen within the muscular layer and in the lamina propria of human postpartum Fallopian tube. The association of telocytes with the microvessels was also observed.

8. LIMITATIONS AND HIGHLIGHTS

Limitations of the study:

1. There was no baseline data available on human non-pregnant Fallopian tube for the variables studied. So the study results could not be compared.

Highlights of the study:

1. Presence of microplicae-like structures in the secretory epithelium of the reproductive age group Fallopian tubes.
2. Presence of clear cell variant of ciliated cell in postpartum Fallopian tube.
3. Presence of high endothelial venules in postpartum Fallopian tube and transendothelial migration of lymphocytes in HEVs.
4. Significant positive correlation between HEVs and IELs.
5. Association of neutrophil with HEVs.
6. Presence of interstitial cells of Cajal within the wall of arteriole and venule.
7. Association of telocytes with blood vessels

9. FUTURE SCOPE OF THE STUDY

1. Microvasculature of non-pregnant Fallopian tube has to be quantified to get the baseline data on human Fallopian tube.
2. As HEVs are exclusively for lymphocyte migration, the association of neutrophils with HEVs demonstrated both under light and electron microscopy warrants further study.
3. The types of intraepithelial lymphocytes should be characterized based on specific markers for cytotoxic T cells, helper T cell and B cell lineage using immunohistochemistry for a better understanding of their role in the immune tolerance and immune surveillance functions of the Fallopian tube.
4. Increase in the number of mast cells in the human postpartum Fallopian tube could possibly be a factor for postnatal infertility. Study of the mast cell numbers and phenotype changes in tubal ectopic pregnancy and non pregnant Fallopian tube could be done.
5. The clear cell variant of ciliated cell that was seen consistently in the human postpartum Fallopian tube has to be further explored.

10. REFERENCES

1. Nutu M. The role of progesterone in the regulation of ciliary activity in the fallopian tube [Internet]. Institute of Neuroscience and Physiology. Department of Physiology; 2009 [cited 2014 Sep 3]. Available from: https://gupea.ub.gu.se/handle/2077/21415

2. Standring S. Gray's Anatomy : The Anatomical Basis of Clinical Practice. 40th Edition. London: Elsevier; 2008. 1292-1293 p.

3. Eroschenko,Victor P. diFIORE'S ATLAS OF HISTOLOGY WITH FUNCTIONAL CORRELATIONS. 12th edition. Lippincot Williams & Wilkins;

4. Ross MH, Pawlina W. Histology: A Text and Atlas, with Correlated Cell and Molecular Biology. 6TH EDITION. Lippincott Williams & Wilkins: Wolters Kluwer; 2011.

5. Jansen RP. Fallopian tube isthmic mucus and ovum transport. Science. 1978 Jul 28;201(4353):349–51.

6. Young B, S Lowe J, Stevens A, W Heath J. Female reproductive system. WHEATER'S - Functional Histology A text and colour atlas. 5th edition. Elsievier; 2006. p. 367–8.

7. Tripathy SN. The Fallopian Tubes. JP Medical Ltd; 2013. 233 p.

8. C. Henrikson R, I. Kaye G, E. Mazurkiewicz J. NMS Histology. New Delhi, India: B.I. Waverly Pvt. Ltd., New Delhi; 1998.

9. Nutu M. The role of progesterone in the regulation of ciliary activity in the fallopian tube [Internet]. Institute of Neuroscience and Physiology. Department of Physiology; 2009 [cited 2014 Jun 17]. Available from: https://gupea.ub.gu.se/handle/2077/21415

10. Chang MC. Development and fate of transferred rabbit ova or blastocyst in relation to the ovulation time of recipients. J Exp Zool. 1950 Jun 1;114(1):197–225.

11. Rodriguez-Martinez H, Tienthai P, Suzuki K, Funahashi H, Ekwall H, Johannisson A. Involvement of oviduct in sperm capacitation and oocyte development in pigs. Reprod Camb Engl Suppl. 2001;58:129–45.

12. Hunter RH. Pre-ovulatory arrest and peri-ovulatory redistribution of competent spermatozoa in the isthmus of the pig oviduct. J Reprod Fertil. 1984 Sep;72(1):203–11.

13. Bhattacharya JKS& S. A Text Book of Immunology. Academic Publishers; 2006. 512 p.

14. Kracke A, Hiller AS, Tschernig T, Kasper M, Kleemann WJ, Tröger HD, et al. Larynx-associated lymphoid tissue (LALT) in young children. Anat Rec. 1997 Jul;248(3):413–20.

15. Knop N, Knop E. Conjunctiva-associated lymphoid tissue in the human eye. Invest Ophthalmol Vis Sci. 2000 May;41(6):1270–9.

16. Knop E, Knop N. Lacrimal Drainage–Associated Lymphoid Tissue (LDALT): A Part of the Human Mucosal Immune System. Invest Ophthalmol Vis Sci. 2001 Mar 1;42(3):566–74.

17. Taeda Y, Ariga N, Okamura K, Takei N, Komeno T, Ueki H, et al. Primary breast mucosa-associated lymphoid tissue (MALT) lymphoma with high-grade transformation evidenced by prominent lymphoepithelial lesions. Breast Cancer Tokyo Jpn. 2006;13(3):322–7.

18. Lee SC, Hong SW, Lee YS, Jeong JJ, Nam K-H, Chung WY, et al. Primary thyroid mucosa-associated lymphoid tissue lymphoma; a clinicopathological study of seven cases. J Korean Surg Soc. 2011 Dec;81(6):374–9.

19. Brandtzaeg P. Mucosal immunity in the female genital tract. J Reprod Immunol. 1997 Nov 30;36(1-2):23–50.

20. Mescher A. Junqueira's Basic Histology, 12th Edition: Text and Atlas, 12th Edition. McGraw Hill Professional; 2009. 480 p.

21. Cesta MF. Normal Structure, Function, and Histology of Mucosa-Associated Lymphoid Tissue. Toxicol Pathol. 2006 Aug 1;34(5):599–608.

22. Wiernsperger N, R. Rapin J. Microvascular Diseases: Is A New Era Coming? Cardiovasc Hematol Agents Med Chem. 2012 Apr 1;10(2):167–83.

23. W. Ham A, H. Cormack D. HAM'S HISTOLOGY. 8th edition. USA: J.B. Lippincott Company, Philadelphia and Toronto; 1950.

24. W. Fawcett D, P. Jensh R. Bloom & Fawcett's Concise Histology. 2nd edition. London: Arnold, Hodder Headline group; 2002.

25. M. Copenhaver W, E. Kelley D, L. Wood R. Bailey's Textbook of HISTOLOGY. 17th edition. USA: Willaims & Wlkins Company; 1978.

26. Boundless. Capilaries - Arteries. Boundless [Internet]. 2014 Jun 27 [cited 2014 Aug 4]; Available from: https://www.boundless.com/physiology/the-cardiovascular-system-blood-vessels/arteries/capilaries/

27. Von Andrian UH, Mempel TR. Homing and cellular traffic in lymph nodes. Nat Rev Immunol. 2003 Nov;3(11):867–78.

28. Anderson AO, Anderson ND. Lymphocyte emigration from high endothelial venules in rat lymph nodes. Immunology. 1976;31(5):731.

29. Girard J-P, Moussion C, Förster R. HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. Nat Rev Immunol. 2012 Nov;12(11):762–73.

30. Girard JP, Springer TA. High endothelial venules (HEVs): specialized endothelium for lymphocyte migration. Immunol Today. 1995 Sep;16(9):449–57.

31. Gowans JL, Knight EJ. The Route of Re-Circulation of Lymphocytes in the Rat. Proc R Soc B Biol Sci. 1964 Jan 14;159(975):257–82.

32. Anderson ND, Anderson AO, Wyllie RG. Specialized structure and metabolic activities of high endothelial venules in rat lymphatic tissues. Immunology. 1976;31(3):455.

33. Stein BA, O'Neill C. Morphometric evidence of changes in the vasculature of the uterine tube of mice induced by the 2-cell embryo on the second day of pregnancy. J Anat. 1994 Oct;185(Pt 2):397–403.

34. Otsuki Y, Maeda Y, Magari S, Sugimoto O. Lymphatics and lymphoid tissue of the fallopian tube: immunoelectronmicroscopic study. Anat Rec. 1989 Dec;225(4):288–96.

35. Seth S, Oberdörfer L, Hyde R, Hoff K, Thies V, Worbs T, et al. CCR7 essentially contributes to the homing of plasmacytoid dendritic cells to lymph nodes under steady-state as well as inflammatory conditions. J Immunol Baltim Md 1950. 2011 Mar 15;186(6):3364–72.

36. Liu K, Victora GD, Schwickert TA, Guermonprez P, Meredith MM, Yao K, et al. In vivo analysis of dendritic cell development and homeostasis. Science. 2009 Apr 17;324(5925):392–7.

37. Chen S, Kawashima H, Lowe JB, Lanier LL, Fukuda M. Suppression of tumor formation in lymph nodes by L-selectin-mediated natural killer cell recruitment. J Exp Med. 2005 Dec 19;202(12):1679–89.

38. Coy P, Garcia-Vázquez FA, Visconti PE, Avilés M. Roles of the oviduct in mammalian fertilization. Reprod Camb Engl. 2012 Oct 1;

39. Croxatto HB, Ortiz ME. Egg transport in the fallopian tube. Gynecol Invest. 1975;6(3-4):215–25.

40. Halbert SA, Tam PY, Blandau RJ. Egg transport in the rabbit oviduct: the roles of cilia and muscle. Science. 1976 Mar 12;191(4231):1052–3.

41. Spilman CH, Harper MJ. Effects of prostaglandins on oviductal motility and egg transport. Gynecol Invest. 1975;6(3-4):186–205.

42. Halbert SA, Becker DR, Szal SE. Ovum transport in the rat oviductal ampulla in the absence of muscle contractility. Biol Reprod. 1989 Jun;40(6):1131–6.

43. Verco CJ, Gannon BJ, Jones WR. Microvascular architecture of the pregnant rabbit oviduct. Acta Anat (Basel). 1984;118(3):167–70.

44. Johnson AD. The oviduct and its functions. Elsevier; 2012. 390 p.

45. Shaw JLV, Dey SK, Critchley HOD, Horne AW. Current knowledge of the aetiology of human tubal ectopic pregnancy. Hum Reprod Update. 2010 Aug;16(4):432–44.

46. Abe H. The mammalian oviductal epithelium: regional variations in cytological and functional aspects of the oviductal secretory cells. Histol Histopathol. 1996 Jul;11(3):743–68.

47. Mondéjar I, Martínez-Martínez I, Avilés M, Coy P. Identification of Potential Oviductal Factors Responsible for Zona Pellucida Hardening and Monospermy During Fertilization in Mammals. Biol Reprod. 2013 Sep 1;89(3):67.

48. Critoph FN, Dennis KJ. The cellular composition of the human oviduct epithelium. Br J Obstet Gynaecol. 1977 Mar;84(3):219–21.

49. Donnez J, Casanas-Roux F, Ferin J, Thomas K. Changes in ciliation and cell height in human tubal epithelium in the fertile and post-fertile years. Maturitas. 1983 Jun;5(1):39–45.

50. Lyons RA, Saridogan E, Djahanbakhch O. The reproductive significance of human Fallopian tube cilia. Hum Reprod Update. 2006 Jul 1;12(4):363–72.

51. Verhage HG, Bareither ML, Jaffe RC, Akbar M. Cyclic changes in ciliation, secretion and cell height of the oviductal epithelium in women. Am J Anat. 1979 Dec;156(4):505–21.

52. Seki K, Rawson J, Eddy CA, Smith NK, Pauerstein CJ. Deciliation in the puerperal fallopian tube. Fertil Steril. 1978 Jan;29(1):75–83.

53. Correr S, Makabe S, Heyn R, Relucenti M, Naguro T, Familiari G. Microplicae-like structures of the fallopian tube in postmenopausal women as shown by electron microscopy. Histol Histopathol. 2006 Mar;21(3):219–26.

54. Frappart L, Berger G, Bethouart M, Palayer C, Bremond A, Rochet Y, et al. [Tubal epithelium. Scanning electron microscopy in the menstrual cycle, in pregnancy and in the menopause (author's transl)]. J Gynécologie Obstétrique Biol Reprod. 1980;9(3):307–13.

55. Perrot-Applanat M, Groyer-Picard MT, Garcia E, Lorenzo F, Milgrom E. Immunocytochemical demonstration of estrogen and progesterone receptors in muscle cells of uterine arteries in rabbits and humans. Endocrinology. 1988 Sep;123(3):1511–9.

56. Perrot-Applanat M, Groyer-Picard MT, Garcia E, Lorenzo F, Milgrom E. Immunocytochemical Demonstration of Estrogen and Progesterone Receptors in Muscle Cells of Uterine Arteries in Rabbits and Humans. Endocrinology. 1988 Sep 1;123(3):1511–9.

57. Gordon JD, Mesiano S, Zaloudek CJ, Jaffe RB. Vascular endothelial growth factor localization in human ovary and fallopian tubes: possible role in reproductive

function and ovarian cyst formation. J Clin Endocrinol Metab. 1996 Jan;81(1):353–9.

58. Verco CJ, Gannon BJ, Jones WR. Fallopian tube microvasculature in the rabbit. Aust J Exp Biol Med Sci. 1983 Feb;61(Pt 1):127–38.

59. Yamamoto S, Konishi I, Tsuruta Y, Nanbu K, Mandai M, Kuroda H, et al. Expression of vascular endothelial growth factor (VEGF) during folliculogenesis and corpus luteum formation in the human ovary. Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol. 1997 Dec;11(6):371–81.

60. Reynolds LP, Redmer DA. Expression of the angiogenic factors, basic fibroblast growth factor and vascular endothelial growth factor, in the ovary. J Anim Sci. 1998 Jun;76(6):1671–81.

61. Kutteh WH, Blackwell RE, Gore H, Kutteh CC, Carr BR, Mestecky J. Secretory immune system of the female reproductive tract. II. Local immune system in normal and infected fallopian tube. Fertil Steril. 1990 Jul;54(1):51–5.

62. Kutteh WH, Mestecky J. Secretory immunity in the female reproductive tract. Am J Reprod Immunol N Y N 1989. 1994 Jan;31(1):40–6.

63. Morris H, Emms M, Visser T, Timme A. Lymphoid tissue of the normal fallopian tube--a form of mucosal-associated lymphoid tissue (MALT)? Int J Gynecol Pathol Off J Int Soc Gynecol Pathol. 1986;5(1):11–22.

64. Kutteh WH, Blackwell RE, Gore H, Kutteh CC, Carr BR, Mestecky J. Secretory immune system of the female reproductive tract. II. Local immune system in normal and infected fallopian tube. Fertil Steril. 1990 Jul;54(1):51–5.

65. Boehme M, Donat H. Identification of lymphocyte subsets in the human fallopian tube. Am J Reprod Immunol N Y N 1989. 1992 Sep;28(2):81–4.

66. Ulziibat S, Ejima K, Shibata Y, Hishikawa Y, Kitajima M, Fujishita A, et al. Identification of estrogen receptor β-positive intraepithelial lymphocytes and their possible roles in normal and tubal pregnancy oviducts. Hum Reprod. 2006 Sep 1;21(9):2281–9.

67. Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. Science. 1996 Apr 5;272(5258):60–6.

68. Girard J-P, Springer TA. High endothelial venules (HEVs): specialized endothelium for lymphocyte migration. Immunol Today. 1995 Sep;16(9):449–57.

69. Kawashima H, Hirakawa J, Tobisawa Y, Fukuda M, Saga Y. Conditional gene targeting in mouse high endothelial venules. J Immunol Baltim Md 1950. 2009 May 1;182(9):5461–8.

70. Warnock RA, Campbell JJ, Dorf ME, Matsuzawa A, McEvoy LM, Butcher EC. The role of chemokines in the microenvironmental control of T versus B cell arrest in Peyer's patch high endothelial venules. J Exp Med. 2000 Jan 3;191(1):77–88.

71. Csencsits KL, Jutila MA, Pascual DW. Nasal-Associated Lymphoid Tissue: Phenotypic and Functional Evidence for the Primary Role of Peripheral Node Addressin in Naive Lymphocyte Adhesion to High Endothelial Venules in a Mucosal Site. J Immunol. 1999 Aug 1;163(3):1382–9.

72. Xu B, Wagner N, Pham LN, Magno V, Shan Z, Butcher EC, et al. Lymphocyte Homing to Bronchus-associated Lymphoid Tissue (BALT) Is Mediated by L-selectin/PNAd, ?4?1 Integrin/VCAM-1, and LFA-1 Adhesion Pathways. J Exp Med. 2003 May 19;197(10):1255–67.

73. Haynes RJ, Tighe PJ, Scott RA, Singh Dua H. Human conjunctiva contains high endothelial venules that express lymphocyte homing receptors. Exp Eye Res. 1999 Oct;69(4):397–403.

74. Bloom GD. A short history of the mast cell. Acta Oto-Laryngol Suppl. 1984;414:87–92.

75. Crivellato E, Beltrami CA, Mallardi F, Ribatti D. Paul Ehrlich's doctoral thesis: a milestone in the study of mast cells. Br J Haematol. 2003;123(1):19–21.

76. A. Wenk, P. Mast cells. Mikro-Graf Off Publ Mich Soc Histotechnol. 2012;41(2):3–6.

77. Menzies FM, Shepherd MC, Nibbs RJ, Nelson SM. The role of mast cells and their mediators in reproduction, pregnancy and labour. Hum Reprod Update. 2010 Oct 19;dmq053.

78. Theoharides TC, Kempuraj D, Tagen M, Conti P, Kalogeromitros D. Differential release of mast cell mediators and the pathogenesis of inflammation. Immunol Rev. 2007 Jun;217:65–78.

79. Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev. 1997 Oct 1;77(4):1033–79.

80. Petrovic D, Zorc M, Zorc-Pleskovic R, Vraspir-Porenta O. Morphometrical and stereological analysis of myocardial mast cells in myocarditis and dilated cardiomyopathy. Folia Biol (Praha). 1999;45(2):63–6.

81. Bot I, de Jager SCA, Zernecke A, Lindstedt KA, van Berkel TJC, Weber C, et al. Perivascular mast cells promote atherogenesis and induce plaque destabilization in apolipoprotein E-deficient mice. Circulation. 2007 May 15;115(19):2516–25.

82. Sugamata M, Ihara T, Uchiide I. Increase of activated mast cells in human endometriosis. Am J Reprod Immunol N Y N 1989. 2005 Mar;53(3):120–5.

83. Weidinger S, Mayerhofer A, Frungieri MB, Meineke V, Ring J, Kohn FM. Mast cell-sperm interaction: evidence for tryptase and proteinase-activated receptors in the regulation of sperm motility. Hum Reprod Oxf Engl. 2003 Dec;18(12):2519–24.

84. Hemadi M, Nikbakht R, Saharkhiz N. Ketotifen, a mast cell blocker improves sperm motility in asthenospermic infertile men. J Hum Reprod Sci. 2013;6(1):19.

85. Schill WB, Schneider J, Ring J. The use of ketotifen, a mast cell blocker, for treatment of oligo- and asthenozoospermia. Andrologia. 1986 Dec;18(6):570–3.

86. Yamamoto M, Hibi H, Miyake K. New treatment of idiopathic severe oligozoospermia with mast cell blocker: results of a single-blind study. Fertil Steril. 1995 Dec;64(6):1221–3.

87. Sivridis E, Giatromanolaki A, Agnantis N, Anastasiadis P. Mast cell distribution and density in the normal uterus — metachromatic staining using lectins. Eur J Obstet Gynecol Reprod Biol. 2001 Sep;98(1):109–13.

88. Sandvei R, Wollen AL, Flood PR, Anker C. Mast cells in the tubal wall in women using an intrauterine contraceptive device. Br J Obstet Gynaecol. 1986 Jul;93(7):758–64.

89. Kumar V, Abbas AK, Aster JC. Robbins Basic Pathology. Elsevier Health Sciences; 2012. 925 p.

90. Preza GC, Tanner K, Elliott J, Yang OO, Anton PA, Ochoa M-T. Antigen-Presenting Cell Candidates for HIV-1 Transmission in Human Distal Colonic Mucosa Defined by CD207 Dendritic Cells and CD209 Macrophages. AIDS Res Hum Retroviruses. 2014 Mar;30(3):241–9.

91. Macey MR. Antigen presenting cells [Internet]. UpToDate. 2014 [cited 2014 Aug 23]. Available from: http://www.uptodate.com/contents/antigen-presenting-cells

92. Iijima N, Thompson JM, Iwasaki A. Dendritic cells and macrophages in the genitourinary tract. Mucosal Immunol. 2008 Nov;1(6):451–9.

93. Segerer SE, Müller N, van den Brandt J, Kapp M, Dietl J, Reichardt HM, et al. Impact of female sex hormones on the maturation and function of human dendritic cells. Am J Reprod Immunol N Y N 1989. 2009 Sep;62(3):165–73.

94. Popescu LM, Ciontea SM, Cretoiu D, Hinescu ME, Radu E, Ionescu N, et al. Novel type of interstitial cell (Cajal-like) in human fallopian tube. J Cell Mol Med. 2005 Jun;9(2):479–523.

95. Cretoiu SM, Cretoiu D, Marin A, Radu BM, Popescu LM. Telocytes: ultrastructural, immunohistochemical and electrophysiological characteristics in human myometrium. Reprod Camb Engl. 2013 Apr;145(4):357–70.

96. Popescu LM, Faussone-Pellegrini M-S. TELOCYTES - a case of serendipity: the winding way from Interstitial Cells of Cajal (ICC), via Interstitial Cajal-Like Cells (ICLC) to TELOCYTES. J Cell Mol Med. 2010 Apr;14(4):729–40.

97. Zhou J, Zhang Y, Wen X, Cao J, Li D, Lin Q, et al. Telocytes accompanying cardiomyocyte in primary culture: two- and three-dimensional culture environment. J Cell Mol Med. 2010 Nov;14(11):2641–5.

98. Cretoiu SM, Cretoiu D, Simionescu AA, Popescu LM. Telocytes in human fallopian tube and uterus express estrogen and progesterone receptors. Sex Steroids. 2012;91–114.

99. Popescu LM. The tandem: telocytes–stem cells. Int J Biol Biomed Eng. 2011;5(2):83–92.

100. Popescu LM, Nicolescu MI. Telocytes and Stem Cells. Resident Stem Cells and Regenerative Therapy [Internet]. Elsevier; 2013 [cited 2014 May 16]. p. 205–31. Available from: http://linkinghub.elsevier.com/retrieve/pii/B9780124160125000116

101. Okuda Y, Okamura H, Kanzaki H, Kawaguchi K, Suzuki A, Nishimura T. [An ultrastructural study of primary carcinoma of the fallopian tube (author's transl)]. Acta Obstet Gynaecol Jpn. 1980 Dec;32(12):1927–36.

102. Sawicki DW, Choroszewska A, Bem W, Strojny P. Lymphocyte number and distribution in the rat uterine epithelium during estrous cycle and early pregnancy. Cell Tissue Res. 1988 Jul 1;253(1):241–4.

103. Johansson E-L, Rudin A, Wassén L, Holmgren J. Distribution of lymphocytes and adhesion molecules in human cervix and vagina. Immunology. 1999 Feb;96(2):272–7.

104. Hill DJA. Cytokines in Human Reproduction. John Wiley & Sons; 1999. 362 p.

105. Otsuki Y, Maeda Y, Magari S, Sugimoto O. Lymphatics and lymphoid tissue of the fallopian tube: immunoelectronmicroscopic study. Anat Rec. 1989 Dec;225(4):288–96.

106. Abughrien BM, Dore MA, McGeady TA, Fitzpatrick E. Intraepithelial leucocytes in the bovine uterine tube. Cells Tissues Organs. 2000;166(1):20–30.

107. Cho H, Kim YB, No JH, Kim K, Paik JH. Primary extranodal marginal zone B-cell lymphoma of MALT-type involving the fallopian tube. Gynecol Oncol Case Rep. 2012;2(1):6–8.

108. Vesel I. [Alterations in Fallopian tube biopsies caused by pregnancy (author's transl)]. Zentralblatt Für Allg Pathol Pathol Anat. 1975;119(1-2):60–4.

109. Hunt JL, Lynn AAA. Histologic features of surgically removed fallopian tubes. Arch Pathol Lab Med. 2002 Aug;126(8):951–5.

110. Harris B, Klein R, Jerosch-Herold M, Hoffman EA, Ahmed FS, Jacobs DR Jr, et al. The Association of Systemic Microvascular Changes with Lung Function and Lung Density: A Cross-Sectional Study. PLoS ONE. 2012 Dec 20;7(12):e50224.

111. Fowler MJ. Microvascular and Macrovascular Complications of Diabetes. Clin Diabetes. 2008 Apr 1;26(2):77–82.

112. Chang CJ, Chou YY, Lee YS. Electron microscopic studies of microvasculature and sympathetic nerve fibers in dilated cardiomyopathy. Chin Med J (Engl). 1998 Oct;111(10):929–33.

113. Biedka M, Makarewicz R, Marszałek A, Sir J, Kardymowicz H, Goralewska A. Labeling of microvessel density, lymphatic vessel density and potential role of proangiogenic and lymphangiogenic factors as a predictive/prognostic factors after radiotherapy in patients with cervical cancer. Eur J Gynaecol Oncol. 2012;33(4):399–405.

114. Boundless. Capillary Dynamics - Capillary Exchange. Boundless [Internet]. 2014 Jul 2 [cited 2014 Aug 4]; Available from: https://www.boundless.com/physiology/the-cardiovascular-system-blood-vessels/capillary-exchange/capillary-dynamics/

115. Verco CJ. Mammalian oviduct vasculature and blood flow. Arch Biol Med Exp (Santiago). 1991;24:229–229.

116. Marchesi VT, Gowans JL. THE MIGRATION OF LYMPHOCYTES THROUGH THE ENDOTHELIUM OF VENULES IN LYMPH NODES: AN ELECTRON MICROSCOPE STUDY. Proc R Soc Lond Ser B Contain Pap Biol Character R Soc G B. 1964 Jan 14;159:283–90.

117. Sainte-Marie G, Guay G, Peng FS. Association of neutrophils with high endothelial venules in the neonatal rat lymph nodes: a probable relation to immunoincompetence. Acta Anat (Basel). 1995;152(1):41–8.

118. Vidal MTA, de Oliveira Araújo IB, Gurgel CAS, Pereira FDAC, Vilas-Bôas DS, Ramos EAG, et al. Density of mast cells and microvessels in minor salivary gland tumors. Tumour Biol J Int Soc Oncodevelopmental Biol Med. 2012 Oct 20;

119. El-Karaksy A, Mostafa T, Shaeer OK, Bahgat DR, Samir N. Seminal mast cells in infertile asthenozoospermic males. Andrologia. 2007 Dec 1;39(6):244–7.

1. ANNEXURE

I. Proforma-1

II. Proforma-2

III. Proforma-3

IV. Patient information sheet and consent forms

(A) English

(B) Tamil

V. IRB approval

Annexure I

Transmission electron microscopic study of the microvasculature of the human postpartum Fallopian tube

Proforma for quantification of microvessels

Date: Patient Name:

Sample No: Hospital Number:

Block No :

Each field = 50000µm^2.

Field number with Area	Arterioles	Capillaries	HEVs	Venules
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
Total (1mm^2)				

Annexure II

Transmission Electron Microscopic study of microvasculature of human postpartum Fallopian tubes

Proforma for quantification of Intraepithelial lymphocytes

Date: Patient Name:

Sample: Hospital Number:

Block number:

3 / 4 consecutive fields = 1 mm

S.No.	Fields	No. of cells
1		
2		
3		
4		
5		
6		
7		

S.No.	Fields	No. of cells
8		
9		
10		
11		
12		
13		
14		

S.No.	Fields	No. of cells
15		
16		
17		
18		
19		
20		

S.No.	Fields	No. of cells
21		
22		
23		
24		
25		

Annexure III

Transmission electron microscopic study of the microvasculature of postpartum human Fallopian tubes

Proforma for quantification of mast cell

Date: Patient name:

Sample: Hospital number:

Block : Block:

Square number	Number of Mast cells
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	

Square number	Number of Mast cells
26	
27	
28	
29	
30	
31	
32	
33	
34	
35	
36	
37	
38	
39	
40	
41	
42	
43	
44	
45	
46	
47	
48	
49	
50	

Square number	Number of Mast cells
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	

Square number	Number of Mast cells
26	
27	
28	
29	
30	
31	
32	
33	
34	
35	
36	
37	
38	
39	
40	
41	
42	
43	
44	
45	
46	
47	
48	
49	
50	

Annexure – IV (A)

Christian Medical College, Vellore
Department of Anatomy

An Observational study on microvasculature of the postpartum human Fallopian tube under Transmission electron microscope – A pilot study
Patient Information sheet

You are kindly requested to participate in the electron microscopic study of the minute blood vessels of the human fallopian tube. As a part of the surgery (sterilization) you are undergoing, a small segment/portion of Fallopian tube will be removed. That removed portion of Fallopian tube will be collected for the study. Your hospital chart will be referred for information regarding history and clinical examination that has been done on you. This information will be utilized to study the number of small blood vessels in the Fallopian tube. This study also looks for the presence of a specific type of a micro-vessel in the Fallopian tubes and their association with immune cells. Participation in this study will not change the quality of the treatment provided by the consulting doctors.

There is no potential harm or complication that can happen to you by participating in this study.

All details including personal data will be kept highly confidential.

We hope to include about at least 10 people from this hospital in this study in the next 2 years.

Participation in this study is purely voluntary, and that refusal to participate will not involve any penalty or loss of benefits to which you are otherwise entitled.

In case of doubts/ questions, please contact:

1. Dr. Minu Rekha B.,
   Department of Anatomy,
   Christian Medical College,
   Vellore - 632002
   Mobile no: 9750709249

2. Dr. Santhosh Benjamin,
   Department of Obstetrics and Gynaecology,
   Christian Medical College,
   Vellore - 632004

Study ID No: _____

Hospital No: _____

Informed Consent form to participate in an Observational study

Department of Anatomy, Christian Medical College, Vellore

Study Title: Transmission electron microscopic study of the microvasculature of postpartum human Fallopian tube – A pilot study

Participant's name:
Date of Birth / Age (in years):
I_____
_____, wife of _____
(Please tick boxes)
Declare that I have read the information sheet provide to me regarding this study dated _____ and have had the opportunity to ask questions. [ ]

I also understand that my participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my usual treatment or my legal rights [ ]

I understand that the study staff and institutional ethics committee members will not need my permission to look at my health records even if I withdraw from the trial. I agree to this access [ ]

I understand that my identity will not be revealed in any information released to third parties or published [ ]

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose [ ]

I voluntarily agree to take part in this study [ ]

Name:
Signature (or Thumb impression)
of the participant / legally acceptable _____
representative :
Date:

Name of witness:
Relation to participant:
Signature : _____
Date:

Annexure – IV (B)

கிறிஸ்துவ மருத்துவ கல்லூரி, வேலூர்
உடற்கூறியல் துறை

பரப்பு எலக்ட்ரான் நுண்ணோக்கியின் கீழ் மனித கருகுழாயின் (சுபலோபியன் டியூப்) நுண்ணரியலான இரத்த நாளங்களின் ஓர் கண்காணிப்பு ஆய்வு – பைலட் ஆய்வு.

நோயாளியின் தகவல் படிவம்

மனித கருக்குழாயின் மிகச்சிறிய இரத்த நாளங்களை எலக்ட்ரான் நுண்ணோக்கியின் கீழ் கண்காணித்து கணக்கிடும் இந்த ஆய்வில் நீங்கள் பங்குபெருமாறு பணிவுடன் கேட்டுக்கொள்கிறோம்.

நீங்கள் மேற்கொள்ளவிருக்கும் அறுவை சிகிச்சையின் ஒரு பகுதியாக, கரு குழாயின் ஒரு பகுதி நீக்கப்படும். அவ்வாறு நீக்கப்படும் கருகுழாயின் பகுதி இந்த ஆய்விற்காக சேகரிக்கப்படும். மேலும் தங்களின் மருத்துவமனை குறிப்பேட்டிலிருந்து நோய்பற்றிய வரலாறு, மருத்துவ பரிசோதனை பற்றிய தகவல்கள் பயன்படுத்தப்படும். இத்தகவல்கள் குழாயின் சிறிய இரத்த நாளங்களின் எண்ணிக்கையை கணக்கிட பயன்படுத்தப்படும். மேலும் இவ்வாய்வு கருகுழாயில் உள்ள ஓர் குறிப்பிட்ட வகையான நுண்சிறையின் இருப்பையும், நோய் எதிர்ப்பு செல்களுடன் அவைகளின் இணைப்பையும் கண்டறியும் ஆய்வாகும்.

இந்த ஆய்வில் கலந்து கொள்வதினால் எந்தவிதமான தீங்கும் ஏற்படாது. மேலும் தங்களுக்கு அளிக்கப்படும் சிகிச்சையில் எந்தவித மாற்றமும் இருக்காது. தங்களின் தனிப்பட்ட தகவல்கள் உள்ளிட்ட அனைத்து விவரங்களும் மிக நம்பகமான முறையில் பாதுகாக்கப்படும்.

அடுத்த 2 வருடங்களில் இந்த மருத்துவமனையிலிருந்து குறைந்தது 10 பேர் இந்த ஆய்வில் சேர்க்கப்பட உள்ளனர். இந்த ஆய்வில் பங்குபெறுவது முற்றிலும் தங்களின் தனிப்பட்ட விருப்பம். மேலும் இதில் பங்கு பெறுவதாலோ, மறுப்பதாலோ தங்களுக்கு வழக்கமாக அளிக்கப்படும் சிகிச்சைகளோ / சலுகைகளோ மாறுபடாது.

சந்தேகங்கள் / கேள்விகளுக்கு, தொடர்பு கொள்ள வேண்டிய முகவரி.

1. டாக்டர். மினு ரேகா ப.
உடற்கூறியல் துறை
கிறிஸ்துவ மருத்துவக் கல்லூரி
வேலூர் 632 002
தொலைபேசி எண்: 9750709249

2. டாக்டர். சந்தோஷ் ஜோசப் பெஞ்சமின்
மகப்பேறு மகளிர் நோய் மருத்துவயியல் துறை
கிறிஸ்துவ மருத்துவக் கல்லூரி
வேலூர் 632 004

கிறிஸ்துவ மருத்துவக் கல்லூரி, வேலூர்
உடற்கூறியல் துறை

ஆய்வு தலைப்பு :

பரப்பு எலக்ட்ரான் நுண்ணோக்கியின் கீழ் மனித கரு குழாயின் (கூபலோபியன் டியூப்) நுண்ணியலான இரத்த நாளங்களின் ஒர் கண்காணிப்பு ஆய்வு – பைலட் ஆய்வு.

ஒப்புதல் அறிவிப்பு படிவம்

பங்கு பெறுபவரின் பெயர் :

பிறந்த தேதி / வயது :

_____ கணவர் பெயர், _____ என்னும் நான்

1) இந்த ஆய்வுக்கான _____ தேதியிட்ட நோயாளியின் தகவல் படிவத்தை படித்து புரிந்துகொண்டேன். கேள்வி கேட்கும் வாய்ப்பினையும் பெற்றேன் என உறுதியளிக்கிறேன்.

2) இந்த ஆய்வில் பங்குபெறுதல் என் தனிப்பட்ட விருப்பமென்றும் புரிந்துகொண்டேன். மேலும் என் வழக்கமான சிகிச்சையோ அல்லது என் உரிமைகளோ பாதிக்காமல் எந்நேரத்திலும், என் அனுமதியை விலக்கிக் கொள்ளலாம் எனவும் தெரிந்துக்கொண்டேன்.

3) இந்த ஆய்வில் சம்பந்தப்பட்டவர்கள், என்னுடைய நோய் பற்றிய விவரங்களை பயன்படுத்திக்கொள்ள ஒப்புதல் தெரிவிக்கிறேன்.

4) என் நோய் பற்றிய தகவல்கள் அனைத்தும் நம்பகமான முறையில் பாதுகாக்கப்படும் என அறிந்துகொண்டேன்.

5) இந்த ஆய்வின் மூலம் பெறப்படும் தரவு மற்றும் முடிவுகளை எவ்வித தடையுமின்றி அறிவியல் நோக்கத்திற்கு மட்டுமே பயன்படுத்தப்படும் என்பதை ஒப்புக்கொண்டேன்.

மேற்கண்ட ஆய்வில் பங்குபெற நான் தன்னிச்சையாக ஒப்புதல் அளிக்கிறேன்.

பெயர் :

பங்குபெறுபவரின்/சட்டபூர்வமான பிரதிநிதியின்
கையொப்பம்/கைரேகை :

தேதி :

சாட்சியாளரின் பெயர் :
பங்குபெறுபவரின் உறவுமுறை :
கையொப்பம் :
தேதி :

ANNEXURE V

INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE
VELLORE 632 002, INDIA

Dr. B J Prashantham, M.A, M. A., Dr. Min (Clinical)
Director, Christian Counselling Centre
Chairperson, Ethics Committee

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Orth
Chairperson, Research Committee & Principal

Dr. Nihal Thomas
MD,MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edir
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

July 25, 2013

Dr. Minu Rekha
PG Demonstrator
Department of Anatomy
Christian Medical College
Vellore 632 002

Sub: **FLUID Research grant project:**
Transmission electron microscopic study of the microvasculature of the postpartum human Fallopian tube – A pilot study
Dr. Minu Rekha. B, PG Demonstrator, Anatomy, Dr. J. Suganthy,
Dr. Santosh Joseph Benjamin, Obstetrics & Gynaecology, Dr. Visalakshi
Dr. Jayaseelan, Biostatistics.

Ref: IRB Min. No. 8314 [OBSERVE] dated 18.06.2013

Dear Dr. Minu Rekha,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "Transmission electron microscopic study of the microvasculature of the postpartum human Fallopian tube – A pilot study" on June 18, 2013.

The Committees reviewed the following documents:

1. Format for application to IRB submission
2. Patient Information Sheet and Informed Consent Form (English, Tamil and Hindi)
3. Cvs of Drs. Minu Rekha, Suganthy, Santosh Joseph Benjamin, Visalakshi Jayaseelan
4. A CD containing documents 1 - 3

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

Dr. B J Prashantham, M.A, M. A., Dr. Min (Clinical)
Director, Christian Counselling Centre
Chairperson, Ethics Committee

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Orth
Chairperson, Research Committee & Principal

Dr. Nihal Thomas
MD,MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edir
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on June 18, 2013 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Other Affiliations
Dr. Anil Kuruvilla	MBBS, MD, DCH	Professor, Neonatology, CMC.	Internal, Clinician
Dr. Benjamin Perakath	MBBS, MS, FRCS	Professor, Surgery (Colorectal), CMC.	Internal, Clinician
Dr. Chandrasingh	MS, MCH, DMB	Urology, CMC	Internal, Clinician
Dr. Simon Rajaratnam	MBBS, MD, DNB (Endo), MNAMS (Endo), PhD (Endo), FRACP	Professor, Endocrinology, CMC	Internal, Clinician
Dr. Anuradha Bose	MBBS, DCH, MD, MRCP, FRCPCH	Professor, Pediatrics, CMC	Internal, Clinician
Dr. Anup Ramachandran	PhD	The Wellcome Trust Research Laboratory Gastrointestinal Sciences	Internal
Dr. Binu Susan Mathew	MBBS, MD	Associate Professor, Dept. of Clinical Pharmacology	Internal, Pharmacologist
Dr. Ellen Ebenezer Benjamin	M.Sc	Maternity Nursing, CMC	Internal, Nurse
Dr. Rajesh Kannangai	MD, PhD.	Professor & In-charge Retrovirus Laboratory (NRL under NACO), Clinical Virology, CMC	Internal, Clinician
Dr. Ranjith K Moorthy	MBBS M Ch	Professor, Neurological Sciences, CMC	Internal, Clinician

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

Dr. B J Prashantham, M.A, M. A., Dr. Min (Clinical)
Director, Christian Counselling Centre
Chairperson, Ethics Committee

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Orth
Chairperson, Research Committee & Principal

Dr. Nihal Thomas
MD,MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edin
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC	Internal, Clinician
Dr. Ashok Chacko	MD, DM, FRCP, FRCPG, FIMSA, FAMS	Director, Institute of Gastroenterology and Liver Disease, Madras Medical Mission, Chennai	External, Clinician
Dr. Bobby John	MBBS, MD, DM, PHD, MAMS	Cardiology, CMC	Internal, Clinician
Mrs. Pattabiraman	B Sc, DSSA	Social Worker, Vellore	External, Lay Person
Mr. Sampath	B Sc, BL	Advocate	External, Legal Expert
Mr. Joseph Devaraj	B Sc, BD	Chaplain, CMC	Internal, Social Scientist
Dr. B. J. Prashantham (Chairperson), IRB Blue - Internal	MA (Counseling), MA (Theology), Dr Min(Clinical)	Chairperson(IRB)& Director, Christian Counselling Centre, Vellore	External, Scientist
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M. Phil, BL	Sr. Legal Advisor, CMC.	Internal, Legal Expert
Dr. Nihal Thomas	MD MNAMS DNB(Endo) FRACP(Endo) FRCP(Edin)	Secretary IRB (EC)& Dy. Chairperson (IRB), Professor of Endocrinology & Addl. Vice Principal (Research), CMC.	Internal, Clinician

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE
VELLORE 632 002, INDIA

Dr. B J Prashantham, M.A, M. A., Dr. Min (Clinical)
Director, Christian Counselling Centre
Chairperson, Ethics Committee

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Orth
Chairperson, Research Committee & Principal

Dr. Nihal Thomas
MD,MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edir
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

A sum of Rs. 80,000/- (Rupees Eighty Thousand only) for 2 years. A sum of Rs 40,000/- will be sanctioned for 12 months after receipt of the revised proposal, subsequent installment of 40,000/- each will be released at the end of the first year following the receipt of the progress report.

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. J. Suganthy, Department of Anatomy, CMC

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in